DOCTOR OF VETERINARY SCIENCES

On the origin of puppies

A multidisciplinary investigation into Belgian dog breeding facilities

Dendoncker, Pierre-Alexandre

Award date:
2019

Awarding institution:
University of Namur

Pierre-Alexandre Dendoncker

ON THE ORIGIN OF PUPPIES

a multidisciplinary investigation into Belgian dog breeding facilities

GHENT UNIVERSITY

FACULTY OF VETERINARY MEDICINE
accredited by EAEVE

UNIVERSITÉ DE NAMUR

A dissertation submitted to the Universities of Ghent and Namur
in fulfilment of the requirements for the degree of Doctor of Veterinary Sciences (PhD).

On the origin of puppies:

A multidisciplinary investigation into Belgian dog breeding facilities.

Pierre-Alexandre Dendoncker

UGhent Student number: 20031240

UNamur Student number: 20143095

A dissertation submitted to the Universities of Ghent and Namur in fulfilment of the requirements for the degree of Doctor of Veterinary Sciences (PhD).

Academic Year 2018 - 2019

Promotors:

Prof. Dr. Christel Moons

Prof. Dr. Claire Diederich

Prof. Dr. Jeroen Dewulf

Prof. Dr. Tiny De Keuster

Members of the examination committee

Prof. Dr. Hans Nauwynck,
Faculty of Veterinary Medicine, Ghent University, Belgium
President of the committee

Prof. Dr. Filip Van Immerseel
Faculty of Veterinary Medicine, Ghent University, Belgium
Secretary of the committee

Prof. Dr. Etienne Thiry
Faculty of Veterinary Medicine, Liège University, Belgium

Prof. Dr. Edwin Claerebout
Faculty of Veterinary Medicine, Ghent University, Belgium

Prof. Dr. Ann Van Soom
Faculty of Veterinary Medicine, Ghent University, Belgium

Dr. Rachel Casey
Dogs Trust, London, United Kingdom

Dr. Damien Coupeau
Faculty of Sciences, Namur University, Belgium

This research was funded by the Federal Public Service of Health, Food Chain Safety and Environment and by the Flemish Government, Department of Environment.

(Grant number: RT14/01 PUPWELFARE1)

TABLE OF CONTENTS

5-HT: Serotonin

ANOVA: Analysis of variance

AVP: Arginine Vasopressin

BASH: Behavioural assessment and screening of health

C-BARQ: Canine behavioural assessment and research questionnaire

CAV: Canine Adenovirus

CCoV: Canine Corona virus

CDV: Canine Distemper virus

CHV: Canine Herpes virus

CIH: Canine Infectious hepatitis

CPIV: Canine Parainfluenza virus

CPV: Canine Parvo virus

CRCV: Canine Respiratory Coronavirus

DA: Dopamine

E. coli: Eschaerichia coli

ESCCAP: European scientific counsel companion animal parasites

EXC: Excitability

FAVV: Federaal agentschap voor de veiligheid van de voedselketen

GC: Glucocorticoids

HPA: Hypothalamic pituitary adrenal

MD: Mean difference

MDA: Maternally derived antibodies

NSF: Nonsocial fear

O: Oestradiol

OT: Oxytocin

RD: Royal decree

SD: Standard deviation

SNS: Sympathic nervous system

T: Testosterone

TEU: Treaty on European Union

TFEU: Treaty on the functioning of the European Union

TRACES: Trade control and expert system

WHO: World health organisation

WSAVA: World small animal veterinary association

CHAPTER 1:
GENERAL INTRODUCTION

Man's best friend.

This common phrase designating domestic dogs illustrates their success as a social species and could be seen as the desire of humankind for dogs to be creatures that are versatile in fulfilling the most varied tasks. Dogs are bred to fulfil a certain physical and behavioural purpose, sometimes by pushing the phenotypes to the limit. However, the owner's expectation of the dog can diverge from what the dog has to offer. A fluffy, cuddly look can hide a decidedly different behaviour. Additionally, breeders may not take into account the owners' expectation of the dog's behaviour and focus too much on its appearance. Is humankind asking too much of our furry friends? To what extent are the dogs we breed really prepared to be healthy and functional in a household? The thread of this doctoral research is to identify what link one can expect between a dog and its origin.

1. The rise of the pet dog

From early domestication in Late Pleistocene (Larson et al., 2012) to recent days, dogs have been kept and bred to fulfil various tasks, ranging from companionship to production of fur (Wilcox and Walkowicz, 1989). It is very likely that domestication of a canine ancestor occurred in first settlements and early agriculture, and is accepted as a first population bottleneck during which the size of the genetic variation within the population sharply reduced (Freedman and Wayne, 2017; Thalmann et al., 2013). At the dawn of the Victorian era (1837-1901), as a result of the success of dog shows and the urge to breed dogs competitively, strict breeding regimes were implemented (KennelClub, 2018; Thalmann et al., 2013). Because of eugenics, focus of breeding gradually changed from working ability to morphotype (i.e., desired physical characteristics such as size, skull shape, coat colour and texture). Ultimately, this phenotypic selection towards approximately 400 breeds is nowadays considered as second bottleneck (Parker et al., 2004; Sampson and Binns, 2006).

The success of the dog revealed to have a flip side. Until recently, breed standards mainly promoted physical traits (Fédération Cynologique Internationale, 2018), not taking into account health and welfare (e.g., brachycephalic breeds that have trouble breathing) or behavioural traits (e.g., brachycephalic breeds have a truncated behavioural repertoire such as stenosed nostrils and a rudimentary tail which complicate communication through body language) that are inextricably linked to the physical traits. Despite a change in mentality and growing concern over dog welfare (Rooney, 2009), those breed standards have not changed profoundly. They can still be interpreted by breeders and judges in such a way to favour the development of extreme characteristics (extreme morphotype or hypertype) which are causing welfare problems (Guintard and Class, 2017). In parallel, designer dogs (e.g., Labradoodle, Cockapoo) gained popularity (Beverland et al., 2008; Crispin, 2011). These hybrid constructs of purebred parents (e.g., Labrador Retriever X Poodle, American Cocker Spaniel X Miniature Poodle) combine traits of multiple breeds to pursue a desired morphotype. Although outcrossing is a solution for improving the genetic diversity (Leroy, 2011), there are concerns that genetic variability and heterozygosity is not necessarily assessed in designer breeds. First, fixing the hybrid traits into a new breed may lead to new population bottlenecks (Farrell et al., 2015). Second, mutual genetic mutations have been described in Labrador and Poodle or American Cocker Spaniel

and Miniature Poodle, such as progressive rod-cone degeneration (Goldstein et al., 2006). Hence, severe loss of genetic diversity in most dog breeds is a disaster for effective population size (vonHoldt et al., 2010), which represents an idealized size of the dog population based on the loss of the genetic diversity (Husemann et al., 2016). It represents a threat in terms of increased risk of inherited disease, reduced quality of life and lifespan expectations (Lindblad-Toh et al., 2005; Sutter and Ostrander, 2004).

The popularity of dogs did not only result in a shift in breeding purposes, but also increased their economic importance. In western countries, at least 20% of all households keep at least one dog (AVMA, 2012; HAS Kennistransfer & Bedrijfsopleidingen, 2015; STATBEL, 2010). Pet dogs are the motor of a multi-billion-euro industry: food production, veterinary care, speciality services, and naturally, dog breeding (FEDIAF, 2018). In Belgium, breeding and selling of dogs represents 5% of all dog-related incomes, and is an important source of employment (STATBEL, 2010; Vlaamse Overheid dept. L&V, 2013). The breeding methods have also diversified. Not only have breeding kennels grown in size to respond to the increasing demand, international trade arose as profitable business and entrepreneurs from other business fields have been attracted to dog breeding.

Dog breeding on a scale comparable to intensive farming gave rise to concerns by professionals in the field and dog owners, which have often been relayed through popular media. The perception is that, while hobby breeders produce healthy and well socialised dogs that have experienced sufficient socialisation (the process by which puppies learn to relate to conspecifics and other species) and environmental learning (learning which accrues from an engagement with the environment), intensive breeders produce dogs that have more health issues and are less able to function as a companion animal. Also, puppies of foreign origin are often perceived to be of inferior quality compared to locally bred puppies (Schrijver et al., 2015). Additionally, there are indications that puppies originating from intensive breeders and of foreign origin are more at risk for disease outbreaks (Hird et al., 1992; Schumaker et al., 2012; Zicola et al., 2012). The origin of puppies has been linked to an increase in behavioural impairments, bite incidents and, consequently, shelter relinquishment or euthanasia (McMillan et al., 2013; Patronek et al., 1996).

Dog breeders influence the behaviour and health of the puppies they produce, raise or sell on multiple levels. Dog breeders are responsible for the choice of bloodline, and the genetic selection, the environment they shape for the dam and offspring, the care and handling they provide to the puppies, the hygiene they apply in the breeding facility and the prophylactic treatments they perform.

In conclusion, popularity of dogs provoked a shift in breeding practices, giving rise to a societal debate on intensive dog breeding. The next paragraphs focus on current knowledge of behavioural development on one hand and health-management and biosecurity on the other hand.

2. The development of canine behaviour

Behaviour is defined as “the way that an individual behaves in a particular situation or under particular conditions” (CambridgeDictionary, 2018). The development of the individual’s behaviour is the interplay of various factors, shaping the evolution from embryo to fully capable adult. While genome and epigenetic effects set the ground plan, individual development further relays on initial performance (current behaviour), learning processes (future behaviour) and the environment

2.1.The history of learning and memory in dogs

Behaviours of animals have long been investigated. Historically, we’ve come a long way from Aristotle’s scala naturae and Descartes’ automata (Dewsbury, 2003). It is widely acknowledged that animals express certain behaviour not only by genetic predispositions, but also as a result of emotions, learning processes, and memory. Memory involves consolidation and retrieval of acquired information, mainly by learning processes. In comparative psychology and ethology, many kinds of learning have been described in animals, such as habituation, sensitisation, Pavlovian or classical conditioning, Skinnerian, operant or instrumental conditioning, imprinting, habit formation, post-tetanic potentiation, imitation, spatial learning or cognitive mapping, etc. (Churchland, 1986).

A simplistic definition of learning is making the connection between a cue (e.g., the stimulus human provides) and the correct behavioural answer to the cue (e.g., the expected behaviour).

Emotions, learning, or memory, however, cannot be measured, they can only be inferred from behaviour (Cahill et al., 2001).

The behaviour of dogs, and specifically the behavioural development of puppies, has been the subject of numerous studies over the years. A dog's life has been categorised into five periods (Immelmann and Suomi, 1981; Overall, 2013; Scott and Fuller, 1965). The primary or neonatal period ranges from birth to approximately two weeks. Then, a transitional stage ranges to approximately three weeks. The following period covers the third until the fourteenth week and is called the sensitive or socialisation period. The fourth period of development, which lasts until sexual maturation, is called the juvenile period. Last, the dog achieves adult stage. These periods coincide approximately with the progressive maturation and integration of neuronal and hormonal tissues (Fox, 1971; Gross et al., 2010). Overlap between stages among the breeds and individuals within a breed has been described (Landsberg, 2001). While a development model based on stages is simple and widely acknowledged (Piaget, 1977), it may have some limitations. For instance, it does not take into account individual variability (Koolhaas et al., 2010) or neurobiological complexity (Gallistel and Balsam, 2014).

An important goal when breeding companion dogs is to optimally prepare dogs for life in human society, while minimising undesirable behaviours. This behavioural development from puppy to adult companion dog largely depends on socialisation practices and environmental learning. These practices should be appropriate for the age of the dog and begin within a few days of birth to well into adulthood. While it should be aimed at providing exposure to many of the situations and social interactions that the dog is likely to encounter over the course of its life, it is important that it occurs in a controlled and pleasant way (Appleby, 1993).

In socialisation and environmental learning, emphasis is set on a few types of learning (Meunier, 2006). First, habituation and, subsequently, sensitisation, (Thompson and Spencer, 1966) is deemed important in behavioural development of dogs (Appleby, 1993). They result in a decrease of behavioural response (or increase in the case of sensitisation) because of repeated stimulation (Rankin et al., 2009). Second, conditioned reflex is a well known reflexive or automatic type of learning in which a stimulus acquires the capacity to evoke a response that was originally evoked by another stimulus (Pavlov, 1927). Third, positive or negative reinforcement or punishment is a learning method often used in dog training. The dog makes an

association between a particular behaviour and a consequence, which can be a reward or a punishment (Skinner, 1953; Thorndike, 1927). From these processes, Bandura elaborated the Social Learning Theory (Bandura, 1977), which was later renamed Social Cognitive Theory (Bandura, 1986). The two key principles are the mediating processes that occur between stimuli and responses on one hand and the learning of behaviour from the environment through observational learning on the other hand. Dogs also show signs of excitement in response to their achievements, and successful problem-solving may act as reward (McGowan et al., 2014). Learning from conspecifics has been investigated in multiple species (Carpenter and Tomasello, 1995; Gieling et al., 2011; Mason et al., 1984) and has been suggested in dog-human relationships (Merola et al., 2012a, 2012b; Payne et al., 2015).

2.2. A brief summary of the neurophysiology of learning, bonding and coping styles

Research in neurophysiology over the last decades has demonstrated that learning and memory formation is the product of a biochemical sequence of events in the brain (Izquierdo and Medina, 1997). Memory is assumed to be embodied in synaptic and cellular modifications at brain circuits. In this newly formed neural hardware, information is encoded and will be consolidated or modified by future reactivations (Dudai et al., 2015). New neural hardware occurs through life and is not limited to critical periods in early life (Lillard and Erisir, 2011).

Social and nonsocial behaviour are regulated by complex cellular and molecular pathways (Cahill et al., 2001). Neuropeptides, in essence oxytocin (OT), dopamine (DA), serotonin (5-HT), and arginine vasopressin (AVP) have a key-role in the brain circuits modulating motivation, learning and memory. The peptide hormone OT is produced in the hypothalamus, but also in peripheral tissues, and released into the circulatory system and the brain in response to sensory stimulation, e.g., during breastfeeding, labour, sex, but also touch, warmth, and stroking, etc (reviewed by Insel et al., 2010). The OT receptors can be found in the brain and in peripheral regions. Central actions of OT range from the modulation of the neuroendocrine reflexes to the establishment of complex social and bonding behaviours, some of which are related to the reproduction and care of the offspring (reviewed by Gimpl and Fahrenholz, 2001). DA levels continuously signal how good or valuable the current situation is regarding obtaining a reward (Berke, 2018). This signal lets animals decide how vigorously to work toward a goal, while also allowing themselves to learn (Hamid et al., 2016). Serotonin has been found to inhibit

dopamine effects (Olivier et al., 2010), and modulate various processes, such as memory, motor and cognitive abilities, although 5-HT has mainly been studied in response to stressors and anxiety (Koolhaas et al., 2010). Oxytocin and AVP further modulate, mostly by inhibition, processes of learning and memory (Sarnyai and Kovács, 2014). Dopamine, OT and AVP, together with steroid hormones, (i.e., testosterone (T), oestradiol (O) and glucocorticoids (GC)), also have a central role in social behaviour (reviewed by McCall and Singer, 2012). A simplified graphical representation of the endogenous oxytocin system is depicted in figure 1.1.

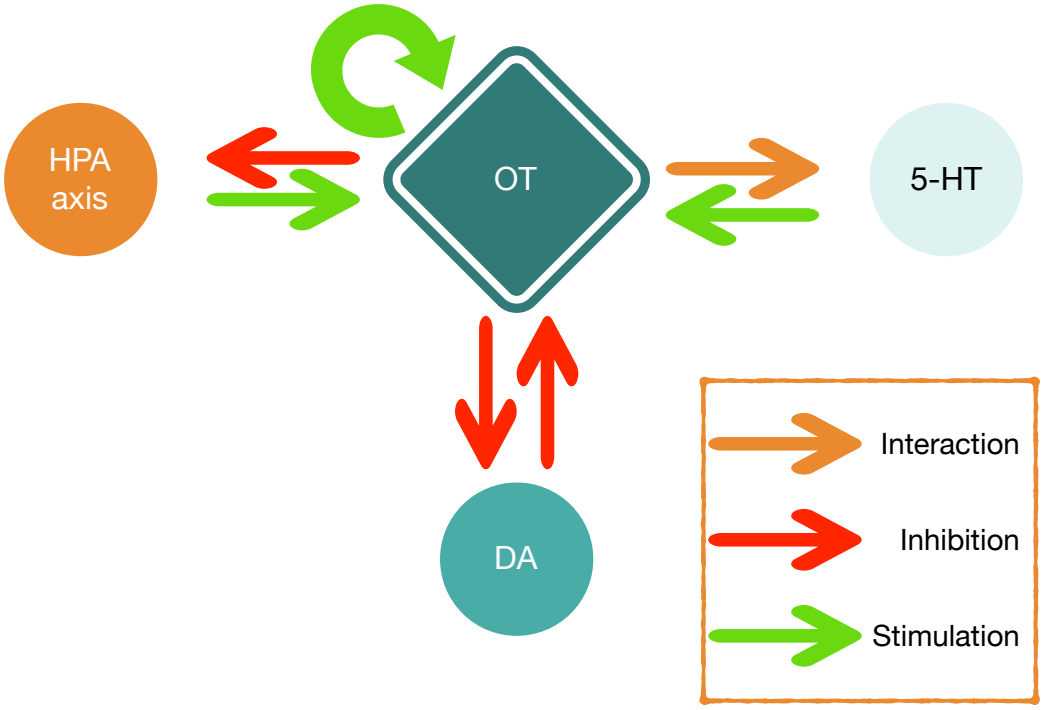

Figure 1.1: Endogenous Oxytocin (OT) system and interactions with Serotonin (5-HT), Dopamine (DA) and Hypothalamic-Pituitary-Adrenal gland (HPA) Axis.

The social bond between the mother and its offspring is probably the most studied social behaviour in mammals, and it is mainly regulated by OT (Kendrick, 2000; Young et al., 2001). In dogs, it is the result of mutual communication between the dam and puppies (Nagasawa et al., 2012; Previde et al., 2009). A strong affectional bond between neonate and the primary caregiver, usually the mother, is important for the survival and future development (Ainsworth, 1969; Bowlby, 1977). Separation from the attachment figure activates the infant's attachment system, which aims at restoring and maintaining proximity with this specific individual (Horn et al., 2013). In humans, the currently reported long-term stability of peripheral OT suggests that

OT represents a 'trait-like' dimension of the individual that probably organises in early childhood and can serve as a whole-life index of sociability (Feldman et al., 2013). Recent research has shown that similar bonding through OT, eventually resulting in a secure base effect (i.e., a buffer against stress), even exists in the dog-human dyad, generally directed at the owner but eventually at unfamiliar humans (Horn et al., 2013; Nagasawa et al., 2009).

Above mentioned neuropeptides (Insel, 2010) and steroid hormones (Nelson and Trainor, 2007) are not only critical to affiliation, cognition and emotion. They also account for aggression, anxiety and stress across a wide variety of species. There are two main stress-responsive systems in the body: the sympathetic nervous system (SNS) and the hypothalamic-pituitary-adrenal (HPA) axis. The SNS is associated with epinephrine and norepinephrine, which exert rapid effects described as "fight or flight", but also have effects on learning and memory (Sullivan et al., 1989). The response to a stressor is also controlled by the HPA axis. Bidirectional relationships of the HPA axis with the serotonergic system and the SNS further complicates this biological picture (Ebner and Singewald, 2017). The HPA-axis triggers the release of stress coping hormones (i.e., GC), which enables animals to cope with threatening or demanding situations (Sapolsky et al., 2000). However, frequently or chronically elevated GC may have detrimental consequences for immunity, welfare, emotions (Protopopova, 2016), and learning and memory processes (McEwen and Sapolsky, 1995). This endocrinological pathway of stress has been shown to be absent in the first four weeks of a dog's life (Nagasawa et al., 2014).

Differences in the regulation of cellular and molecular pathways account for large variances in cognition (Kis et al., 2015), bonding (Romero et al., 2014) and, more generally, behaviour. They account not only for species-specific behaviour (Donaldson and Young, 2008), but also for intraspecific variation (Ebner and Singewald, 2017). As a result, individuals of the same species will present different ways of coping with stressors (Koolhaas et al., 2010). In the past, coping strategies were classified into proactive (the dog reacts actively to a stressor) and reactive (the dog reacts passively to a stressor) (Koolhaas et al., 1999), but there is evidence that individuals can also present an ambivalent behaviour in a social threatening situation (Horváth et al., 2007). In dogs, it has been suggested that coping style is important to welfare in stressful conditions (Corsetti et al., 2018) and that parallels exist between aggressiveness and the proactive

behaviour style, and fearfulness and the reactive coping style (Riemer et al., 2013). Last, there is evidence that coping is not fixed at birth; early life experiences can further modulate coping attitude later in life (Foyer et al., 2013).

3. The effect of domestication and selection on behavioural development

A dog's genome is the result of phylogenetic processes (the evolution of a species). As a result of domestication and millennia of mutualistic relationships between dogs and humans (Miklósi and Topál, 2013), contemporary dogs, compared to their closest relatives, the wolves, are more motivated to interact (Gácsi et al., 2005) and to bond with humans (Topál et al., 2005). Evidence shows that their cognitive social skills allow them to read and follow human social cues, such as verbal commands or gesticulation, or emotions (Wobber and Hare, 2009). Some aspects of these social-cognitive abilities have converged with human social cues (e.g., pointing at, looking at) through a phylogenetic process of enculturation (Hare et al., 2002).

The establishment of breed standards not only led to major changes in the conformation of breeds, some of which can be observed when comparing bloodlines of show dogs and working dogs (Bateson, 2010), but also additional selection towards breed-dependent behaviour has occurred (Spady and Ostrander, 2008). There is evidence that morphotype influences how individuals of a breed behave and communicate. For instance, brachycephalic breeds were scored higher on persistent barking and compulsive staring (McGreevy et al., 2013) and their repertoire of social signalling was decreased compared to their non-brachycephalic conspecifics (Kerswell et al., 2010). Incidences of problematic behaviour have also been suggested to be breed-dependent (Martínez et al., 2011); higher scores for aggression directed towards humans were reported for dachshund and chihuahua (Duffy et al., 2008; Hsu and Sun, 2010). More traits were compared between certain breeds or breed groups (Eken Asp et al., 2015). Basenjis, for instance, showed higher reactivity and lower trainability compared to American cocker spaniels (Scott and Fuller, 1965). Interestingly, geographical differences in breed-dependent behaviour exist, probably because of genetic variation caused by reproductive isolation (Bradshaw and Goodwin, 1999). Breed-dependent behaviours may not solely rely on genetic differences, however. The reported associations may also be the product of prejudice towards a particular breed (e.g., the personality description of breed standards),

acting as a self-fulfilling prophecy (Serpell et al., 2014). They may also be the result of varying owner-perceptions (Beverland et al., 2008) and therefore, different socialisation and husbandry (Bradshaw and Goodwin, 1999).

Paradoxically, although dogs of modern times are mainly kept for companionship, the current breeding standards' main focus is not directed towards providing an emotionally balanced and adapted pet dog (King et al., 2012). The standards are still based on phenotypic selection by Mendelian inheritance of morphotype (Fédération Cynologique Internationale, 2018), hence they are intrinsically arbitrary and mostly imprecise (Bateson, 2010). A more complex inheritance has long been suspected for behavioural traits. In the past, breeding programmes for working dogs with rigorous standardised phenotyping (Svartberg, 2002; Wilsson and Sundgren, 1998a) and quantitative genetic approaches have already demonstrated genetic progress (Goddard and Beilharz, 1983, 1982). Similar improvements might be expected for the selection of companion animals (van Rooy et al., 2014). One avenue of behavioural research aims at identifying variations in specific genes which contribute to individual differences in social behaviour and cognition, and to target those who were linked to developmental disorders (Kumsta et al., 2013). Several association studies have shown that allelic variations in genes involved in OT signaling result in different levels of plasma OT and therefore are important in explaining individual differences in behavioural phenotypes, e.g., polymorphism of the OT receptor relates empathy and stress reactivity in humans, or social organization in birds (reviewed by Chini et al., 2014). Further research in canine-specific genetic factors of neurobiology in general, and breed-dependent differences in particular, is needed.

4. Early-life environment of dogs influences the behavioural development

The importance of the prenatal and post-natal early life environment has been widely studied in multiple species, such as in rats (Klein and Rager, 1995), in monkeys (Clarke and Schneider, 1997), in guinea pigs (Sachser and Kaiser, 1996), in blue foxes (Braastad, 1998) or in dogs (reviewed by Dietz et al., 2018). In the following paragraphs, we provide an overview of research on the influence of the prenatal and postnatal environment shaped by the breeder (i.e., until homing) on the behavioural development of puppies.

4.1. Epigenetic effects on behavioural development

Epigenetic events alter expression for different copies of the same gene in a given cell nucleus. These events provoke heritable changes in gene activity that are not brought about by changes in the DNA sequence (Van Soom et al., 2014). One of the most described epigenetic mechanism is DNA methylation, where transcriptional factors are blocked from gaining access to the gene, effectively silencing expression of the gene (Kumsta et al., 2013). This stable modification is maintained after cell division and will be transferred to next cell generations (Meaney, 2001).

Lack of control in stressful situations during late pregnancy, for example exposure to noise in rhesus monkeys (Clarke et al., 1994), social stress or crowding in rats (Dahlöf et al., 1978; P. R. Lee et al., 2007), or restraint in pigs (Tuchscherer et al., 2002), increases the GC levels of the mother. In rodents, this leads to changes in gene expression (i.e., differential methylation of the glucocorticoid region in hippocampal tissue) and life-long phenotypic differences in physiology and behaviour, including neuroendocrine stress responsivity (i.e., increased HPA axis response), fear-related behaviour and attentional processes, synaptogenesis and cognitive development, and reproductive behaviour and maternal care in female offspring (reviewed by Zhang and Meaney, 2010). DNA methylation is also a pathway whereby early life adversity might produce enduring neurobiological alteration (Cushing and Kramer, 2005). Literature has described long-lasting effects in dogs from early life experiences (pre- or postnatal), but research investigating canine epigenetic effects during pregnancy or early-life is scarce. However, similar neurobiological pathways and epigenetic effects can be expected. More research is needed in order to determine if these reported long-lasting effects may be epigenetic effects.

4.2. Prenatal environment

As described above, developmental alteration in neurobiological systems (as early as in utero) can have functional consequences later in life. Animals subjected to prenatal stress may have a reduced ability to cope with challenging environments, while they may have increased propensity for developing behavioural disturbances and reduced welfare in stressful situations. In dogs, however, research is scarce and results appear controversial, especially considering personality consistency over time (Fratkin et al., 2013). Housing dams in a stressful environment

(i.e., in isolation with minimal human contacts) during the last third of pregnancy has indeed been shown to affect the puppies' learning capacities when tested at 8 weeks of age, but those differences were no longer found one year later. On the contrary, more social enrichment (1h of gentle petting per day) during the last third of the pregnancy was shown to have no effect (Leroy et al., 2009).

4.3. Postnatal environment

The postnatal environment of dogs, was intensively studied in the 1950s and 1960s, mainly by researchers at the Roscoe B. Jackson Memorial Laboratory (Freedman et al., 1961; Scott and Fuller, 1965). This resulted in above mentioned periods of development, that coincide approximately with the progressive maturation and integration of neuronal and hormonal tissues.

At birth, puppies have limited motor abilities and eye and ear canals are closed. During the first weeks of life, puppies are predominantly guided by olfactory and tactile stimuli (Landsberg, 2001). In neonate rats, olfaction is involved in early associative learning and memory and has important role in maternal attachment (Wilson and Sullivan, 1994). Also in rats, tactile stimulation from the dam (i.e., maternal licking and grooming, mainly of urogenital region) directly regulates physiology and affects neuronal development in offspring (Liu et al., 1997). Recent literature investigating the effect of early-life experiences and environment (i.e., genetic and hormonal) on canine behaviour is scarce, however, effects similar to the ones described in rodents can be expected. Recent animal and human studies show that aversive situations in the first weeks have lasting effects on serotonergic function in adulthood. Early maternal separation in rats (J.-H. Lee et al., 2007) or monkeys (Clarke et al., 1994) and childhood abuse in humans (Penza et al., 2003) can result in major changes in 5-HT responses (i.e., epigenetic effects of the 5-HT transporter gene results in decreased availability of 5-HT) and alteration of the HPA-axis, sensitising these individuals for stressors later in life.

Other maternal effects influencing behavioural development of puppies have been described. A higher parity of the dam may positively influence the maturation of puppies and, indirectly, their boldness in play or exploration (Wilsson and Sundgren, 1998b). The dam is a secure base for the puppies (Previde et al., 2009), and distressed mothers may not only alter the socialisation

capabilities of their offspring, but also negatively influence exploration and environmental learning (Range et al., 2007). The progressive estrangement of the mother, accompanied by gradual weaning, generally starts around week 4 post-partum (Fox, 1972), but complete maternal separation in six-week-old dogs was shown to have a negative effect on physical conditions and impair the bonding process with humans, compared with dogs that had maternal contact until 12 weeks (Slabbert and Rasa, 1993). During the weaning process, an increasing interaction between the mother and her offspring is seen, involving more aggressive and more social interactions (Wilsson, 1984).

The breeding dam is not the only determinant of behavioural development, and other factors, which can be influenced by the breeder, have been investigated. First, a litter-effect was observed in German Shepherds' future personality, such as aggressiveness, playfulness or curiosity (Strandberg et al., 2005). Two litter factors have been associated with higher occurrence of future problematic behaviour (i.e., anxieties), namely a high number of littermates (Foyer et al., 2013) and early-separation of littermates at four weeks of age (Pierantoni et al., 2011). Interestingly, the moment of rehoming, combined with the maternal separation and complete weaning, is also an important factor of behavioural development. Delayed homing (>12 weeks) was associated with a higher occurrence of unwanted behaviour later in life, not only for puppies raised in a monotonous environment, which provides less stimuli (Scott and Fuller, 1965), but also for those raised in a household and kept with the dam and littermates until 13-16 weeks (Jokinen et al., 2017).

Dog-human interactions during early life were also investigated. Freedman and colleagues (1961) showed that puppies that had limited interaction with humans at age 5–9 weeks did not exhibit any fear response to a handler and could be trained easily, while puppies kept in isolation until the age of 14 weeks remained fearful despite subsequent careful handling and petting (Freedman et al., 1961). Interestingly, there is evidence that the human caretaker gains importance when poor maternal care is present (Gazzano et al., 2008), which could be explained because mother-infant bond does not only exist between a dam and her offspring but can also appear between a primary caretaker (a foster animal or a human) and a nursling (Gácsi et al., 2013). Puppies given increased socialisation were less fearful towards humans later in life (Hubrecht, 1995). Although similar association was suggested by a recent cross-

sectional owner directed survey (Tiira and Lohi, 2015), not all socialisation programmes tend to result in long-term effects (Batt et al., 2010).

The effect of resources and nonsocial environmental aspects on behavioural development were also investigated. Puppies raised on corrugated cardboard were observed to be less active compared to the ones raised on insulating soft pile blankets (Wilsson and Sundgren, 1998b). The type of food and feeding regimen had a positive effect on growth and subsequently, positively influenced performance in a behavioural test (Wilsson and Sundgren, 1998b). Toys are generally accepted as a viable enrichment of the environment. However, beneficial effects on behavioural development are not straightforward. First, not all toys provide the same value of mental and physical stimuli (Wells, 2004). Second, the effect of toys is limited compared to contact with caretakers (Hubrecht, 1995), therefore it must not be seen as a replacement social enrichment. Appropriate toys increase the complexity of dog behaviour, substantially change the expression of behaviour and help to prevent undesirable behaviours, however, a decrease in socialising with kennel mates has been described in adult laboratory dogs (Hubrecht, 1993). Additionally, social and nonsocial learning programmes providing diverse novel stimuli did not find beneficial effect on the behavioural development (Batt et al., 2010; Seksel et al., 1999).

In conclusion, the environment shaped by the breeder can contribute on multiple levels to the behavioural development of young puppies. Adverse situations at the breeder will probably result in behavioural impairment later in life. Although the benefits of providing diverse stimuli early in life to achieve better socialisation and environmental learning are commonly discussed anecdotally, empirical studies providing evidence for long-term beneficial effects are scarce. More research surrounding canine behavioural development and the influence of various environments is necessary.

5. Health issues during puppy development

Infectious diseases are caused by pathogens, which include bacteria, fungi, protozoa, worms, viruses, and even infectious proteins called prions (Alberts et al., 2002). Infection, however, does not necessarily lead to disease. Only when the pathogen has successfully established a site of infection in the host disease does occur (Janeway et al., 2001). The incidence of disease among those infected varies greatly depending on the individual susceptibility of the host but

also on the particular pathogen (e.g., the invasiveness). Spread and persistence of pathogens is impacted by the host (i.e., species, immune status) and the environment (e.g., temperature, humidity, presence of vectors etc.) (Scholthof, 2007).

5.1. The development of immunity

The foetal development of canine lymphoid tissue has been described and an immune response of a puppy is already possible in the final trimester of gestation (Day, 2007). Due to endotheliochorial placentation, passive transfer of immunity (immunoglobulin) in utero is very limited (Stoffel et al., 2000). When born, all puppies have the constitutive components of a functional, but naive immune system in place. Maternal immunity is transferred by drinking colostrum during the first 24 hours of life. After ingestion, maternal immunoglobulin will decline exponentially at a rate that is probably breed-dependent (Chappuis, 1998). There may be considerable variation between littermates in the efficiency of uptake of colostral immunoglobulin, and variation in the concentration of specific antibodies within the colostrum is also expected between individual dams (Day, 2007). Meanwhile, endogenous production of immunoglobulins starts by week 2 (Toman et al., 2002). Although maternal immunity is essential to new-born puppies (Root Kustritz, 2011), high concentrations of maternal immunoglobulins inhibit endogenous neonatal immune response. In practice, the point at which endogenous production relays the maternal immunoglobulin one is vaguely circumscribed as between 6 and 12 weeks (Day, 2007), although recent evidence suggest. During the following months, immunity will further differentiate. Although dogs are immunocompetent at birth, their immunity reaches full maturity by one to two years of age, depending on the breed (Faldyna et al., 2001).

5.2. Infectious diseases of importance during early life

Numerous pathogens leading to canine diseases have been identified, some of which affect multiple hosts. To a certain extent, they will have an impact on animal welfare, public health and economic perspectives (Cleaveland et al., 2001). Pathogens commonly affecting puppies are described below.

5.2.1. Common canine helminths, protozoa, and ectoparasites

Intestinal parasites (i.e.,, protozoa and helminths) are common pathogens in dogs of all ages, often causing a loss of condition by inducing malabsorption, vomiting and anaemia in larger infestations (Robertson et al., 2000). The prevalence of enteric parasites for puppies with different origins have been investigated worldwide: e.g., in Australia (Bugg et al., 1999), Belgium (Claerebout et al., 2009; Dupont et al., 2013), Japan (Itoh et al., 2011, 2009, 2005), the Netherlands (Overgaauw and Boersema, 1998) Romania (Mircean et al., 2012), and the United States of America (Stehr-Green and Murray, 1987). Although values vary depending on the setting (veterinary clinic, pet store, breeding kennel, shelter), relatively high prevalences are reported for protozoa such as Giardia spp. (ranging between 9.3% and 43.9 %) and coccidia (i.e., Cystoisospora spp., ranging between 2.0% and 26.3%). Except for Toxocara canis (ranging between 4.4% and 26.3 %), low prevalences for most helminths (i.e., Ancylostoma caninum and Dipylidium caninum) are reported (Claerebout et al., 2009). Large breeding kennels and pet stores are considered a risk factor for parasitic infection (Dupont et al., 2013; Itoh et al., 2011, 2005). Interestingly, puppies are more often infected with enteropathogens than adult dogs, a direct result of their immature immune system and environmental conditions (higher animal density, doubtful hygiene). (Fontanarrosa et al., 2006; Kirkpatrick, 1988; Ramírez-Barrios et al., 2004). The decrease of prevalence in relation to age is a combination of the maturating immunity on one hand and the weaning and rehoming on the other hand, resulting in the disappearance of the transmission route (placental for T. canis, transmammary for Ancylostoma spp.). The zoonotic risk of *T. canis* has been widely acknowledged (Overgaauw and van Knapen, 2013).

Other commonly seen parasites in puppies are ectoparasites that affect the skin. Fleas, or Ctenocephalides felis, feed on the blood of dogs and cats and sometimes humans (reviewed by Dobler and Pfeffer, 2011). In Belgium, it is the most common ectoparasite found on dogs (Diez et al., 2015). While infestation is mainly linked with itching, flea allergy dermatitis can occur in dogs that are allergic to flea saliva (Wilkerson et al., 2004). Compulsive scratching and biting can also lead to skin trauma and secondary bacterial infections. Furthermore, the flea is intermediate host of Dipylidium caninum (Beugnet et al., 2014). The environment, and especially the traffic of animals and humans, is an important factor influencing the larval stage (Robinson,

1995). Thorough treatment of all susceptible animals and the environment is necessary to control flea infestations.

Scabies or Sarcoptic mange, caused by Sarcoptes scabiei, is a very contagious parasitic skin disorder in most mammals. Sarcoptes mites burrow through the skin which causes severe itchiness and leads to self-inflicted trauma and secondary bacterial skin infections (reviewed by Arlian and Morgan, 2017). Environmental contamination is a source of scabies in most mammals (Arlian et al., 1989). Dogs suffering from scabies not only have diminished welfare, they may also represent a zoonotic risk.

Demodex canis is an ectoparasite of dogs, living in small numbers in the hair follicle (Fondati et al., 2010). As long as the dogs' immune system functions properly (Healey and Gaafar, 1977), the mites cause no mange. The Demodex mites are transmitted to puppies from their mother during the first few days of life (Gortel, 2006). Young puppies, with immature immune systems are most susceptible to demodectic mange, and this disease therefore occurs primarily in dogs younger than two years (Tsai et al., 2011).

Cheyletiellosis, caused by Cheyletiella spp mites, is seen in dogs, cats, rabbits, and humans. Because mites can be seen macroscopically as they crawl across skin and fur and cause itching and scaling, infestation is also referred to as 'walking dandruff' (reviewed by Curtis, 2004). Cheyletiellosis spreads quickly in breeding kennels, and the mites can also spread to humans (Dodd, 1970).

Otodectes cynotis, or ear mites, live mostly on the surface of the ear canal of dogs, cats, rabbits, and ferrets (reviewed by Curtis, 2004). Infestations are common in puppies, although any age can be affected (Salib and Baraka, 2011). Treatment should be thorough and not only include all susceptible animals, but also the environment as this parasite is relatively stable in the environment and highly contagious (Otranto et al., 2004).

5.2.2. Common canine bacteria

Bacteria show a high prevalence in diverse canine populations. For instance, Escherichia coli and Clostridium perfringens are ubiquitous because they are part of the normal canine adult intestinal microflora. A high prevalence is difficult to interpret since not all strains may be

implicated in the development of clinical signs such as diarrhoea or fever (Hall 2004). The periparturient period is undoubtedly the period with the highest risk of disease transmission, and bacterial infectious diseases are a frequent cause of neonatal death in dogs (Münnich and Küchenmeister, 2014). E. coli, Staphylococcus sp., streptococci, and to a lesser extent Bordetella bronchiseptica, Klebsiella sp., and Salmonella spp. are reported as common causative factors of canine morbidity and puppy mortality (Münnich and Lübke-Becker, 2004; Nielen et al., 1998; Schäfer-Somi et al., 2003). Additionally, kennel cough, a respiratory disease mainly caused by B. bronchiseptica, eventually in combination with canine parainfluenza virus and canine adenovirus, is regularly observed in breeding facilities (Buonavoglia and Martella, 2007).

5.2.3. Common canine viruses

Several viruses such as canine distemper virus (CDV), canine corona virus (CCoV) and canine parvovirus-2 (CPV) are common pathogens of domestic and wild carnivores and have a worldwide distribution (Decaro et al., 2011; Patel and Heldens, 2009). CDV is a Morbillivirus (family Paramyxoviridae) that is very resistant to cool temperatures but quickly inactivated by ultraviolet light and by heat and drying. It is transmitted by aerosols or contact with oral, respiratory, and ocular fluids and exudates containing the virus. Therefore, dense populations of susceptible animals are needed to sustain epidemics (Acosta-Jamett et al., 2015; Martella et al., 2008).

CPV is a major global pathogen of dogs and is related to high morbidity and mortality (Gordon and Angrick, 1986; Miranda and Thompson, 2016; Truyen, 1999). This non-enveloped virus (family Parvoviridae) is very resistant in the environment, e.g., it is able to survive up to 6 months at room temperature (Eterpi et al., 2009). It is transmitted by faecal-oral route, probably mainly through ingestion of virus from the environment, rather than by direct contact with infected animals. The presence of CPV has been reported in wild and domestic canid populations and strains are observed to cross the species barrier (Sobrino et al., 2008). A CPV variant, type 2c, emerged in the previous decade and is circulating worldwide along with the former CPV types 2a and 2b (Decaro et al., 2007; Decaro and Buonavoglia, 2012).

Since the first report of CCoV, several CCoV outbreaks have been reported worldwide, emphasising that CCoV is an important pathogen of the dog (Decaro et al., 2011; Decaro and Buonavoglia, 2011, 2008a; Ellis et al., 2005; Naylor et al., 2001; Stavisky et al., 2010). Serological and virological investigations have demonstrated that CCoV is widespread in dog populations, mainly in kennels and animal shelters (Bandai et al., 1999; Pratelli et al., 2001; Rimmelzwaan et al., 1991; Schulz et al., 2008), and the presence of CCoV has been suggested as a marker for the success of biosecurity measures within the facility (Stavisky et al., 2012). CCoV infection is characterised by high morbidity (i.e., gastrointestinal) and low mortality, through a typical faecal–oral route of transmission (Decaro and Buonavoglia, 2008a). Except for the hyper-virulent CCoV strains (Decaro and Buonavoglia, 2008b), CCoV is usually regarded as a mild canine pathogen. Mortality of CCoV commonly occurs as a consequence of mixed infections with CPV (Decaro and Buonavoglia, 2008a; Pratelli et al., 2001) or canine adenovirus type 1 (Decaro et al., 2007). A newer genotype of CCoV was only detected in 2003 (Pratelli, 2006). This genotype leads to an emerging systemic and fatal infection and is called pantropic canine coronavirus (Zicola et al., 2012).

Additional viruses represent an increased risk for breeding kennels. Canine infectious hepatitis is making an uprising in Italy, in relation with dog import (Decaro et al., 2007), while some viruses impact reproduction and neonates, such as the canine herpesvirus and minute virus of canines (Decaro et al., 2008; Ronsse et al., 2005, 2002).

5.3. Transmission of pathogens

Profound knowledge and understanding of the different transmission pathways provide guidance for the treatment and prevention of diseases. Pathogens are most often spread through direct contact. This is the result of direct contact between dogs or by means of droplets. A proper designed housing with adequate management will make it possible to limit and control direct contact transmission.

Transmission of pathogens between dogs can be classified into two categories: direct and indirect. Direct transmission between dogs is without doubt the most common one. It is the result of the animal flow and the contact possibilities between dogs and the infection path of pathogens. Although the risk of pathogen introduction in a breeding facility through semen is

much smaller, transmission can also occur by venereal transmission (i.e., brucellosis, canine herpesvirosis, venereal tumours, leishmaniosis, toxoplasmosis) (Silva et al., 2009).

Indirect contact transmission mainly involves inanimate objects called fomites that become contaminated by pathogens from an infected individual. In dogs, the faecal-oral route is a very common form of indirect transmission. Additionally, some pathogens can be spread through vectors such as insects and ticks (Beugnet and Marié, 2009), rodents (Jansen et al., 2005), birds (Song et al., 2008), other domestic animals (Crawford et al., 2005), and feral animals (Kapel et al., 2006). Finally, transmission may also occur indirectly through mechanical vectors like vehicles, staff, and visitors. Transmission paths are inherent to a pathogens' ability to survive in the environment and vice-versa. Particularly non-enveloped viruses (e.g., Canine Parvovirus), mycobacteria, bacterial spores and coccidian oocysts can survive for months and are largely resistant to disinfectants (McDonnell and Russell, 1999).

5.4. Environmental measures to limit health issues

The sum of measures taken to reduce the entrance, persistence and transmission of pathogens can be defined as biosecurity (Anderson, 1998). Biosecurity has been a subject of research in production animals such as poultry, pig farms and cattle since the end of the nineties (Dewulf and Van Immerseel, 2018). The main reason lays in the shift from individual medicine at animal level to disease prevention at facility level (Sarrazin et al., 2014; Villarroel et al., 2007). However, the use of the term biosecurity is unusual in the field of companion animals. Although veterinarians have collaborated with shelters and kennels for decades, until recently, little scientific interest existed in the discipline of preventive companion animals' veterinary medicine in these settings. Recent publications describing importance of biosecurity in dog facilities and providing applicable measures are directed towards shelters (Newbury and Miller, 2018), veterinary clinics (Amory et al., 2010) or temporary gatherings such as contests or exhibitions (Stull et al., 2016) and breeding kennels (Dendoncker et al., 2018). The interest in this field is growing, as the need for expertise in risk assessment and disease management is recognised (Miller and Zawistowski, 2013).

External biosecurity on one hand consists of all preventive measures to counter the entrance of pathogens into the facility. The health status and susceptibility to infectious disease of newly

acquired animals may represent a threat to the health of other dogs in the breeding facility. The purchase policy is an important risk factor for the entrance of pathogens in livestock facilities (Filippitzi et al., 2017). Historically, quarantine has been accepted as the best measure to limit the risks of entrance for pathogens of interest (Gensini et al., 2004).

Internal biosecurity on the other hand includes the measures to restrain the spread of pathogens between animals within a facility, and is also referred to as biocontainment (Villarroel et al., 2007). The facility's design can assist to a great extent in transmission reduction if it allows segregation. This segregation is also referred to as compartmentalisation. It consists of the separation of animals into different groups or subpopulations based on their vulnerability to infectious diseases and health status with respect to a specific disease (Scott et al., 2006). It is also the measure of choice to counter outbreaks in a facility (Hurley, 2009). All movements (of animals, humans, equipment, etc.) between compartments must be strictly restricted.

Puppies and pregnant dams are more at risk to contract a disease. The environment can harbour various pathogens that will impair their health and welfare or represent a zoonotic risk. Appropriate strategies, including environmental factors, are of utmost importance to limit disease. More research surrounding the influence of various environments shaped by dog breeders on the spread of pathogens is necessary.

6. Origin of puppies: does it matter?

A popular belief among future dog owners is that the choice of the breeder is of utmost importance when acquiring a puppy. Various motivators for dog breeding can be found on the world wide web, ranging from passion to profit and everything in between. Intensive dog breeding systems have been called puppy mills (U.S. District Court for the District of Minnesota, 1984), puppy farms or less responsible breeders (Crispin, 2011). International puppy trade on the other hand, has been associated with criminal activities (Wyatt et al., 2016). Questionnaires were developed for owners to recognise these breeders (Gray et al., 2016). However, scientific criteria to determine the responsibility of a breeder are weak. For instance: there is no evidence of a threshold number of dog breeds produced by the breeder below which a certain quality is guaranteed. There is also no evidence that the presence of the mother at the age of homing is associated with less behavioural problems or health issues. In Belgium,

selling and breeding of dogs is mainly regulated by animal welfare governments. However, a systematic investigation of the environment shaped by various types of dog breeders is currently lacking.

6.1. Legal context of dog breeding in Belgium

At the onset of this research, multiple bills regulated nationwide dog-related activities, such as dog breeding. First, all dogs are to be identified and registered in the official database called DogID (KB 25/04/2014). Second, the transport of dogs is regulated (KB 29/10/2014). Third, specific regulation applies regarding the prevention and control of rabies (KB 17/10/2016). Last, the modalities to breed, house and sell puppies are regulated by specific legislation. The different breeder types are regulated by the Law on Animal Welfare of 14 August 1986 (chapter III) and by the bill defining the conditions for the approval of establishments for animals and the conditions for the marketing of animals (RD 27/04/2007, modified by RD 14/09/2007, by RD 18/03/2009, and by RD 15/11/2010). Four types of dog breeders are defined: occasional breeder (up to two litters a year), hobby breeder (up to 10 litters a year), professional breeder and breeder-merchant (more than 10 litters a year). All breeders need to apply for accreditation, except occasional breeders. Additional subdivision of accredited breeders into small or large hinges on having equal to or less, respectively more, than 10 females on site. This regulation results in seven categories: occasional breeders, small hobby breeders, large hobby breeders, small professional breeders, small breeder merchants and large breeder merchants. Breeder merchants largely consist of former pet shops who, subsequent to above mentioned bill, have been forced to add dog breeding to their activities in order to be able to continue to sell puppies from other breeders. Breeder merchants are the only ones allowed to sell dogs sourced from other breeders. These external sources can be of foreign origin, if the source breeder meets the standards set out in annex 3 of RD 27/04/2007 (i.e., being subjected to the same standards as Belgian breeders). This bill not only covers warranty aspects and housing conditions, but also defines requirements regarding staff, health management, and includes biosecurity measures.

Because of the regionalisation of animal welfare, this bill was further modified in Wallonia (Arrêté Gouvernement Wallon MB 31/05/2017). From June 2017, Walloon occasional breeders are subjected to registration as well. Another regional-specific bill developed by the Flemish

ministry of agriculture, is directed at dog breeders in Flanders to improve genetic diversity and limit hereditary disorders (Besluit Vlaamse Regering MB 19/03/2010 and MB 03/03/2015). The associations and organisations recognised to apply the above are regulated (MB 27/07/2011). All dog breeders with more than ten dogs must apply for an environmental permit in their respective region. The conditions are region-dependent. Additional limitations surrounding dog breeding have been observed on the municipal level.

A database query at the onset of this project revealed that approximately 55% of dogs were registered by private persons (mainly originating from occasional breeders although they could have been registered after rehoming), 20% by hobby and professional breeders, and 25% by breeder-merchants (Welfare, 2014).

Although free movements of persons, goods and services does apply in the Schengen area (Article 3(2) of the Treaty on European Union (TEU); Article 21 of the Treaty on the Functioning of the European Union (TFEU); Titles IV and V TFEU; Article 45 of the Charter of Fundamental Rights of the European Union), the import of dogs is subjected to specific regulations. All dogs must be identified by microchip, accompanied by a European passport, and must have received a valid rabies vaccination. The latter means that travel can occur not earlier than 21 days post rabies primo-vaccination which cannot be administered before 12 weeks of age (EU 576/2013). Additionally, all intra-community movements of dogs associated with a change of ownership are considered commercial movements and must be registered in the European Trade Control and Expert System (TRACES). We further refer to these as commercial imports. In Belgium, transports of less than 6 dogs accompanied by their owner or a legal representative are exempted and are further referred to as parallel import. Additionally, veterinarians in the field received the directive (omzendbrief FAVV 11/02/2015) that they should only report to the authorities illegally imported dogs (i.e., the ones without a certified rabies vaccine or subjected to fraud), when they are the subject of commercial transport, or originate from countries at risk.

At the onset of this doctoral research, nationwide statistics reported 20% of all newly registered dogs (30.209) had a passport issued abroad (Welfare, 2014). Recent statistics of the Flemish Government confirm the importance of import of dogs: out of 98.551 registered dogs in Flanders in 2016, 19.142 (19.4%) were registered as commercial import, with the main countries of origin being Slovakia (n=11975) and Czech Republic (n=4478).

6.2. Sources outside the scope of our research

Although the focus of this doctoral research lays on the differences between registered breeder categories, puppies can also originate from other sources. Puppies obtained by means of parallel import, a shelter or the illegal circuit, such as backyard breeders or illegal import are out of scope of this doctoral research. While parallel import is not a new phenomenon, internet-sales clearly facilitate buying a puppy from abroad and therefore this is not limited to short distances or breeders wanting a dog from a particular bloodline only.

Puppies can be adopted from a shelter, not only locally, but also internationally. Belgian dog-rescuing organisations declare to rehome thousands of dogs yearly (i.e., from Spain, Romania, Greece, Croatia). Although few organisations declare to register dogs in TRACES (e.g., SHIN-ACE), most declare using parallel import into Belgium, by rehoming dogs through the Netherlands (RESCANI VZW), to fly them over with tourists that agree to act as legal representative (Arca Noah España), or to rehome them first in Belgian foster homes (FFSHP VZW). These organisations use different methods, which makes it difficult to demarcate good from bad practices. However, taking into account the scale at which some organisations operate, the associated risks for transport of sheltered animals are not different to the ones of commercial transport. Additionally, the definition of commercial trade however, could be interpreted differently in the near future, since recent changes in the civil code regarding entrepreneurs and enterprises have regrouped all economic activities and now apply to non-profit organisations (RD 11/08/2017 and RD 15/04/2018). It would therefore be preferable to provide a clear legal framework for larger organisations.

The illegal circuit is per definition difficult to study, however, anecdotal reports indicate that it is an important practice, which can be of great concern to animal welfare but also public health. Despite not being included in the scope of this research, it is clear from the scale of these practices that also these routes of acquisition could benefit from closer examination.

6.3. Differences between breeders

There is growing evidence that the conditions of housing, caring, and raising of puppies diverge between breeder categories, however, existing studies are faced with a number of difficulties. First, investigation of environmental differences is mostly performed indirectly and

retrospectively by investigating outcomes in adult dogs (Wauthier et al., 2018), and this is especially the case in behavioural research (McMillan, 2017). Because few studies investigate the environment directly, or focus on a time point that is situated as close as possible to the time at the breeder (i.e., preferably before or right after homing), they not only measure differences attributable to the breeder but also differences related to ownership and the associated context in which the puppy was raised. Second, the breeder-type is often an owner-reported variable collected through convenience sampling instead of a verified variable collected through sampling of official databases. This is an important selection bias because dog owners are mostly unaware of their puppy´s breeders' category unless there is a motivation to investigate the source. Third, the classification of dog breeders tends to be erratic in studies. Commonly, 'breeder category' is being dichotomized, such as registered or unregistered breeder (Korbelik et al., 2011), pet store or other (Hird et al., 1992), responsible or less responsible breeders (Gray et al., 2016), etc. However, this does not necessarily relay the variation in size of the facility, as described by De Meester and colleagues (De Meester et al., 2005). Last, the few surveys designed to describe dog breeding facilities were based on self-declaration only. Although anonymous mail questionnaires have multiple advantages, one cannot rule out social desirability bias (Mortel, 2008).

Because of the assumed importance of the environment on incidence of disease and behavioural development, breeders can impact the health of the puppies they sell, and also influence their early socialisation and environmental learning. In order to study the epidemiology of infectious diseases in dogs, it is important to consider the origin of the surveyed canine populations. Data obtained without considering the variability of the origin may induce apparently significant but biologically meaningless differences among the results (Itoh et al., 2009). Considering the socialisation of a dog, breeders also play an important role. The intra-specific socialisation will occur through contact with the dam, the littermates, and possibly other dogs. Inter-specific socialisation will depend on whether and how the breeder (and subsequently the owner) allows contact with other animal species and humans.

The execution of early socialisation and environmental learning, however, tends to conflict with health management according to widespread popular and veterinarian beliefs (Stepita et al., 2013). Increasing the contact with conspecifics of different ages and the use of various

materials goes against biosecurity principles aiming to limit contacts and control the environment (Dewulf and Van Immerseel, 2018). On the other hand, application of strict biosecurity measures without considering the social behaviour of dogs could result in inadequate management and housing of dogs, which may increase the incidence of acute and chronic stress (Beerda et al., 1999). Chronic stress not only impacts the welfare and the behavioural development of a puppy, it has also been linked to effects on health and immunity. By altering tissue physiology and dampening immune responses to, for instance, invasive pathogens or antimicrobial products such as toxins, chronic stress influences the introduction and spread of infectious diseases (Beerda et al., 1999; Radek, 2010). Moreover, large-scale facilities are prone to disease spread if preventive measures, such as biosecurity procedures, are suboptimal (Schumaker et al., 2012).

In summary, the popularity of dogs has provoked a shift in breeding practices, giving rise to a societal debate on intensive dog breeding. The environment shaped by the breeder is important for the behavioural development of puppies, and adverse situations at the breeder can result in behavioural impairment later in life. In parallel, the environment can harbour various pathogens that will impair health and welfare or represent a zoonotic risk, and puppies and pregnant dams are more at risk to contract a disease. Appropriate strategies, including environmental factors, are of utmost importance to limit disease. Dog breeding facilities tend to vary greatly, and the inferiority of puppies originating from intensive breeding facilities is suggested. However, no systematic investigation of the various breeding systems has been performed. To assess all environmental factors influencing the behavioural development and the health of puppies, a multidisciplinary approach is required.

Meanwhile, guidelines on how to improve socialisation and environmental learning practices without compromising the welfare and health of puppies in intensive dog breeding are currently lacking. It is crucial to characterize the current husbandry conditions at different breeder types that may influence the health, hygiene, socialisation, environmental learning, and welfare. Subsequently, measuring behavioural and health outcomes in relation to the environment will provide potential areas of improvement. Finally, an evaluation of pet dog behaviour shortly after homing will help to assess the differences between dog breeder types, as perceived by the owner after the sale.

7. References

Acosta-Jamett, G., Surot, D., Cortés, M., Marambio, V., Valenzuela, C., Vallverdu, A., Ward, M.P., 2015. Epidemiology of canine distemper and canine parvovirus in domestic dogs in urban and rural areas of the Araucanía region in Chile, Veterinary Microbiology. https://doi.org/10.1016/j.vetmic.2015.05.012

Ainsworth, M.D.S., 1969. Object Relations, Dependency, and Attachment: A Theoretical Review of the Infant-Mother Relationship. Child Dev. 40, 969. https://doi.org/10.2307/1127008

Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., Walter, P., 2002. Introduction to Pathogens.

Amory, H., Clercx, C., Desmecht, D., Dufrasne, I., Gabriel, A., Gommeren, K., Marlier, D., Rollin, F., Thiry, E., Galen, G. Van, Gommeren, K., Saegerman, C., Galen, G. Van, 2010. Biosecurity SOP applied to the Faculty of Veterinary Medicine of the University of Liège Biosecurity Standard Operating Procedures (SOP) applied.

Anderson, J.F., 1998. Biosecurity: A new term for an old concept: How to apply it. Bov. Pract. 32, 61–70.

Appleby, D., 1993. Socialization and habituation, in: The Behavior of Dogs and Cats. London.

Arlian, L.G., Morgan, M.S., 2017. A review of Sarcoptes scabiei: past, present and future. Parasit. Vectors 10, 297. https://doi.org/10.1186/s13071-017-2234-1

Arlian, L.G., Vyszenski-Moher, D.L., Pole, M.J., 1989. Survival of adults and developmental stages ofSarcoptes scabiei var.canis when off the host. Exp. Appl. Acarol. 6, 181–187. https://doi.org/10.1007/BF01193978

AVMA, 2012. U.S. pet ownership and demographics sourcebook. American Veterinary Medical Association, Schaumburg, IL.

Ballweber, L.R., Xiao, L., Bowman, D.D., Kahn, G., Cama, V.A., 2010. Giardiasis in dogs and cats: update on epidemiology and public health significance. Trends Parasitol. 26, 180–189. https://doi.org/10.1016/j.pt.2010.02.005

Bandai, C., Ishiguro, S., Masuya, N., Hohdatsu, T., Mochizuki, M., 1999. Canine coronavirus infections in Japan: virological and epidemiological aspects. J. Vet. 61, 731–736.

Bandura, A., 1986. Social Cognitive Theory of Social Referencing, in: Social Referencing and the Social Construction of Reality in Infancy. Springer US, Boston, MA, pp. 175–208. https://doi.org/10.1007/978-1-4899-2462-9_8

Bandura, A., 1977. Social learning theory. Prentice Hall.

Bateson, P., 2010. Independent Inquiry into Dog Breeding 1–65.

Batt, L., Batt, M., Baguley, J., McGreevy, P., 2010. Relationships between puppy management practices and reported measures of success in guide dog training. J. Vet. Behav. Clin. Appl. Res. 5, 240–246. https://doi.org/10.1016/j.jveb.2010.02.004

Beerda, B., Schilder, M.B.., Bernadina, W., Van Hooff, J.A.R.A.., De Vries, H.W., Mol, J.A., 1999. Chronic Stress in Dogs Subjected to Social and Spatial Restriction. II. Hormonal and Immunological Responses. Physiol. Behav. 66, 243–254. https://doi.org/10.1016/S0031-9384(98)00290-X

Berke, J.D., 2018. What does dopamine mean? Nat. Neurosci. 21, 787–793. https://doi.org/10.1038/s41593-018-0152-y

Beugnet, F., Labuschagne, M., Fourie, J., Jacques, G., Farkas, R., Cozma, V., Halos, L., Hellmann, K., Knaus, M., Rehbein, S., 2014. Occurrence of Dipylidium caninum in fleas from client-owned cats and dogs in Europe using a new PCR detection assay. Vet. Parasitol. 205, 300–306. https://doi.org/10.1016/J.VETPAR.2014.06.008

Beugnet, F., Marié, J.-L., 2009. Emerging arthropod-borne diseases of companion animals in Europe. Vet. Parasitol. 163, 298–305. https://doi.org/10.1016/j.vetpar.2009.03.028

Beverland, M.B., Farrelly, F., Lim, E.A.C., 2008. Exploring the dark side of pet ownership: Status- and control-based pet consumption. J. Bus. Res. 61, 490–496. https://doi.org/10.1016/J.JBUSRES.2006.08.009

Bowlby, J., 1977. The making and breaking of affectional bonds. I. Aetiology and psychopathology in the light of attachment theory. An expanded version of the Fiftieth Maudsley Lecture, delivered before the Royal College of Psychiatrists, 19 November 1976. Br. J. Psychiatry 130, 201–10. https://doi.org/10.1192/BJP.130.3.201

Braastad, B.O., 1998. Effects of prenatal stress on behaviour of offspring of laboratory and farmed mammals. Appl. Anim. Behav. Sci. 61, 159–180. https://doi.org/10.1016/S0168-1591(98)00188-9

Bradshaw, J.W.S., Goodwin, D., 1999. Determination of behavioural traits of pure-bred dogs using factor analysis and cluster analysis; a comparison of studies in the USA and UK. Res. Vet. Sci. 66, 73–76. https://doi.org/10.1053/RVSC.1998.0246

Bugg, R.J., Robertson, I.D., Elliot, A.D., Thompson, R.C.A., 1999. Gastrointestinal Parasites of Urban Dogs in Perth, Western Australia. Vet. J. 157, 295–301. https://doi.org/10.1053/tvjl.1998.0327

Buonavoglia, C., Martella, V., 2007. Canine respiratory viruses. Vet. Res. 38, 355–373. https://doi.org/10.1051/vetres:2006058

Cahill, L., McGaugh, J.L., Weinberger, N.M., 2001. The neurobiology of learning and memory: some reminders to remember. Trends Neurosci. 24, 578–581. https://doi.org/10.1016/S0166-2236(00)01885-3

CambridgeDictionary, 2018. Definition [WWW Document]. URL https://dictionary.cambridge.org/dictionary/english/behaviour

Carpenter, M., Tomasello, M., 1995. Joint Attention and Imitative Learning in Children, Chimpanzees, and Enculturated Chimpanzees*. Soc. Dev. 4, 217–237. https://doi.org/10.1111/j.1467-9507.1995.tb00063.x

Chappuis, G., 1998. Neonatal immunity and immunisation in early age: lessons from veterinary medicine. Vaccine 16, 1468–1472. https://doi.org/10.1016/S0264-410X(98)00110-8

Charles, A.J., Travers, P., Walport, M., Shlomchik, M.J., 2001. Infectious agents and how they cause disease.

Chini, B., Leonzino, M., Braida, D., Sala, M., 2014. Learning About Oxytocin: Pharmacologic and Behavioral Issues. Biol. Psychiatry 76, 360–366. https://doi.org/10.1016/J.BIOPSYCH.2013.08.029

Churchland, P.S., 1986. Neurophilosophy: Toward a Unified Science of the Mind-Brain. The MIT Press, Cambridge, Massachusetts.

Claerebout, E., Casaert, S., Dalemans, A.C., De Wilde, N., Levecke, B., Vercruysse, J., Geurden, T., 2009. Giardia and other intestinal parasites in different dog populations in Northern Belgium. Vet Parasitol 161, 41–46. https://doi.org/10.1016/j.vetpar.2008.11.024

Clarke, A.S., Schneider, M.L., 1997. Effects of Prenatal Stress on Behavior in Adolescent Rhesus Monkeys. Ann. N. Y. Acad. Sci. 807, 490–491. https://doi.org/10.1111/j.1749-6632.1997.tb51947.x

Clarke, A.S., Wittwer, D.J., Abbott, D.H., Schneider, M.L., 1994. Long-term effects of prenatal stress on HPA axis activity in juvenile rhesus monkeys. Dev. Psychobiol. 27, 257–269. https://doi.org/10.1002/dev.420270502

Cleaveland, S., Laurenson, M.K., Taylor, L.H., 2001. Diseases of humans and their domestic mammals: pathogen characteristics, host range and the risk of emergence. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 356, 991–999. https://doi.org/10.1098/rstb.2001.0889

Corsetti, S., Borruso, S., Di Traglia, M., Lai, O., Alfieri, L., Villavecchia, A., Cariola, G., Spaziani, A., Natoli, E., 2018. Bold personality makes domestic dogs entering a shelter less vulnerable to diseases. PLoS One 13, e0193794. https://doi.org/10.1371/journal.pone.0193794

Crawford, P., Dubovi, E., Castleman, W., 2005. Transmission of equine influenza virus to dogs. Science (80-.). 310, 482–485.

Crispin, S., 2011. The Advisory Council on the Welfare Issues of Dog Breeding. Vet. J. 189, 129–131. https://doi.org/10.1016/J.TVJL.2011.06.008

Curtis, C.F., 2004. Current trends in the treatment of Sarcoptes, Cheyletiella and Otodectes mite infestations in dogs and cats. Vet Dermatol 15, 108–114. https://doi.org/10.1111/j.1365-3164.2004.00362.x

Cushing, B.S., Kramer, K.M., 2005. Mechanisms underlying epigenetic effects of early social experience: The role of neuropeptides and steroids. Neurosci. Biobehav. Rev. 29, 1089–1105. https://doi.org/10.1016/J.NEUBIOREV.2005.04.001

Dahlöf, L.-G., Hård, E., Larsson, K., 1978. Influence of maternal stress on the development of the fetal genital system. Physiol. Behav. 20, 193–195.

Day, M.J., 2007. Immune System Development in the Dog and Cat. J. Comp. Pathol. 137, S10–S15.

De Meester, R., Moons, C., van Bree, H., Coopman, F., 2005. Critical evaluation of the environment in Belgian dog breeding kennels during the puppies' socialization period. Vlaams Diergeneeskd. Tijdschr. 74, 364–374.

Decaro, N., Buonavoglia, C., 2012. Canine parvovirus—A review of epidemiological and diagnostic aspects, with emphasis on type 2c. Vet. Microbiol. 155, 1–12. https://doi.org/10.1016/j.vetmic.2011.09.007

Decaro, N., Buonavoglia, C., 2011. Canine coronavirus: not only an enteric pathogen. Vet. Clin. North Am. Small Anim. Pract. 41, 1121–32. https://doi.org/10.1016/j.cvsm.2011.07.005

Decaro, N., Buonavoglia, C., 2008a. An update on canine coronaviruses: Viral evolution and pathobiology. Vet. Microbiol. 132, 221–234. https://doi.org/10.1016/j.vetmic.2008.06.007

Decaro, N., Buonavoglia, C., 2008b. An update on canine coronaviruses: Viral evolution and pathobiology. Vet. Microbiol. 132, 221–234. https://doi.org/10.1016/J.VETMIC.2008.06.007

Decaro, N., Campolo, M., Elia, G., Buonavoglia, D., Colaianni, M.L., Lorusso, A., Mari, V., Buonavoglia, C., 2007. Infectious canine hepatitis: an "old" disease reemerging in Italy. Res Vet Sci 83, 269–273. https://doi.org/10.1016/j.rvsc.2006.11.009

Decaro, N., Desario, C., Addie, D.D., Martella, V., Vieira, M.J., Elia, G., Zicola, A., Davis, C., Thompson, G., Thiry, E., Truyen, U., Buonavoglia, C., 2007. The study molecular epidemiology of canine parvovirus, Europe. Emerg. Infect. Dis. 13, 1222–4. https://doi.org/10.3201/eid1308.070505

Decaro, N., Desario, C., Billi, M., Mari, V., Elia, G., Cavalli, A., Martella, V., Buonavoglia, C., 2011. Western European epidemiological survey for parvovirus and coronavirus infections in dogs. Vet. J. 187, 195–199. https://doi.org/10.1016/j.tvjl.2009.10.027

Decaro, N., Martella, V., Buonavoglia, C., 2008. Canine Adenoviruses and Herpesvirus. Vet. Clin. Small Anim. Pract. 38, 799–814. https://doi.org/10.1016/j.cvsm.2008.02.006

Dendoncker, P.-A., de Rooster, H., Abma, E., Wydooghe, E., Dewulf, J., 2018. Biosecurity measures for dog merchants and canine breeding kennels, in: Dewulf, J., Van Immerseel, F. (Eds.), Biosecurity in Animal Production and Veterinary Medicine : From Principles to Practice. Acco, Leuven / Den Haag, pp. 433–452.

Dewsbury, D.A., 2003. Comparative Psychology, in: Handbook of Psychology. John Wiley & Sons, Inc., Hoboken, NJ, USA, pp. 67–84. https://doi.org/10.1002/0471264385.wei0104

Dewulf, J., Van Immerseel, F., 2018. Biosecurity in animal production and veterinary medicine : From principles to practice., first edit. ed.

Dietz, L., Arnold, A.-M.K., Goerlich-Jansson, V.C., Vinke, C.M., 2018. The importance of early life experiences for the development of behavioural disorders in domestic dogs. Behaviour 155, 83–114. https://doi.org/10.1163/1568539X-00003486

Diez, M., Picavet, P., Ricci, R., Dequenne, M., Renard, M., Bongartz, A., Farnir, F., 2015. Health screening to identify opportunities to improve preventive medicine in cats and dogs. J. Small Anim. Pract. 56, 463–469. https://doi.org/10.1111/jsap.12365

Dobler, G., Pfeffer, M., 2011. Fleas as parasites of the family Canidae. Parasit. Vectors 4, 139. https://doi.org/10.1186/1756-3305-4-139

Dodd, K., 1970. Cheyletiella yasguri: widespread infestation in a breeding kennel., Veterinary Record. British Veterinary Medicine.

Donaldson, Z.R., Young, L.J., 2008. Oxytocin, vasopressin, and the neurogenetics of sociality. Science 322, 900–4. https://doi.org/10.1126/science.1158668

Dudai, Y., Karni, A., Born, J., 2015. The Consolidation and Transformation of Memory. Neuron 88, 20–32. https://doi.org/10.1016/J.NEURON.2015.09.004

Duffy, D.L., Hsu, Y., Serpell, J.A., 2008. Breed differences in canine aggression. Appl. Anim. Behav. Sci. 114, 441–460. https://doi.org/https://doi.org/10.1016/j.applanim.2008.04.006

Dupont, S., Butaye, P., Claerebout, E., Theuns, S., Duchateau, L., Van de Maele, I., Daminet, S., 2013. Enteropathogens in pups from pet shops and breeding facilities. J. Small Anim. Pract. 54, 475–480. https://doi.org/10.1111/jsap.12119

Ebner, K., Singewald, N., 2017. Individual differences in stress susceptibility and stress inhibitory mechanisms. Curr. Opin. Behav. Sci. 14, 54–64. https://doi.org/10.1016/J.COBEHA.2016.11.016

Eken Asp, H., Fikse, W.F., Nilsson, K., Strandberg, E., 2015. Breed differences in everyday behaviour of dogs. Appl. Anim. Behav. Sci. 169, 69–77. https://doi.org/10.1016/J.APPLANIM.2015.04.010

Ellis, J.A., Mclean, N., Haines, D.M., 2005. Detection of coronavirus in cases of tracheobronchitis in dogs: A retrospective study from 1971 to 2003.

Eterpi, M., McDonnell, G., Thomas, V., 2009. Disinfection efficacy against parvoviruses compared with reference viruses. J. Hosp. Infect. 73, 64–70. https://doi.org/10.1016/j.jhin.2009.05.016

Faldyna, M., Levá, L., Knötigová, P., Toman, M., 2001. Lymphocyte subsets in peripheral blood of dogs — a flow cytometric study. Vet. Immunol. Immunopathol. 82, 23–37. https://doi.org/10.1016/S0165-2427(01)00337-3

Farrell, L.L., Schoenebeck, J.J., Wiener, P., Clements, D.N., Summers, K.M., 2015. The challenges of pedigree dog health: approaches to combating inherited disease. Canine Genet. Epidemiol. 2, 3. https://doi.org/10.1186/s40575-015-0014-9

Fédération Cynologique Internationale, 2018. FCI Standards Commission [WWW Document]. URL http://www.fci.be/en/FCI-Standards-Commission-72.html (accessed 12.10.18).

FEDIAF, 2018. European statistics 2017 [WWW Document]. URL http://www.fediaf.org/who-we-are/european-statistics.html (accessed 12.10.18).

Feldman, R., Gordon, I., Influs, M., Gutbir, T., Ebstein, R.P., 2013. Parental oxytocin and early caregiving jointly shape children's oxytocin response and social reciprocity. Neuropsychopharmacology 38, 1154–1162. https://doi.org/10.1038/npp.2013.22

Filippitzi, M.E., Brinch Kruse, A., Postma, M., Sarrazin, S., Maes, D., Alban, L., Nielsen, L.R., Dewulf, J., 2017. Review of transmission routes of 24 infectious diseases preventable by biosecurity measures and comparison of the implementation of these measures in pig herds in six European countries. Transbound. Emerg. Dis. https://doi.org/10.1111/tbed.12758

Fondati, A., Lucia, M. De, Furiani, N., Monaco, M., Ordeix, L., Scarampella, F., 2010. Prevalence of Demodex canis-positive healthy dogs at trichoscopic examination. Vet. Dermatol. 21, 146–151. https://doi.org/10.1111/J.1365-3164.2009.00769.X

Fontanarrosa, M.F., Vezzani, D., Basabe, J., Eiras, D.F., 2006. An epidemiological study of gastrointestinal parasites of dogs from Southern Greater Buenos Aires (Argentina): Age,

gender, breed, mixed infections, and seasonal and spatial patterns. Vet. Parasitol. 136, 283–295. https://doi.org/10.1016/J.VETPAR.2005.11.012

Fox, M.W., 1972. Understanding your dog. Coward, McCann & Geoghegan.

Fox, M.W., 1971. Integrative development of brain and behavior in the dog. University of Chicago Press, Chicago.

Foyer, P., Wilsson, E., Wright, D., Jensen, P., 2013. Early experiences modulate stress coping in a population of German shepherd dogs. Appl. Anim. Behav. Sci. 146, 79–87. https://doi.org/10.1016/J.APPLANIM.2013.03.013

Fratkin, J.L., Sinn, D.L., Patall, E.A., Gosling, S.D., 2013. Personality Consistency in Dogs: A Meta-Analysis. PLoS One 8, e54907. https://doi.org/10.1371/journal.pone.0054907

Freedman, A.H., Wayne, R.K., 2017. Deciphering the Origin of Dogs: From Fossils to Genomes. Annu. Rev. Anim. Biosci. 5, 281–307. https://doi.org/10.1146/annurev-animal-022114-110937

Freedman, D.G., King, J.A., Elliot, O., 1961. Critical period in the social development of dogs. Science 133, 1016–7. https://doi.org/10.1126/SCIENCE.133.3457.1016

Gácsi, M., Győri, B., Miklósi, Á., Virányi, Z., Kubinyi, E., Topál, J., Csányi, V., 2005. Species-specific differences and similarities in the behavior of hand-raised dog and wolf pups in social situations with humans. Dev. Psychobiol. 47, 111–122. https://doi.org/10.1002/dev.20082

Gácsi, M., Maros, K., Sernkvist, S., Faragó, T., Miklósi, Á., 2013. Human Analogue Safe Haven Effect of the Owner: Behavioural and Heart Rate Response to Stressful Social Stimuli in Dogs. PLoS One 8. https://doi.org/10.1371/journal.pone.0058475

Gallistel, C.R., Balsam, P.D., 2014. Time to rethink the neural mechanisms of learning and memory. Neurobiol. Learn. Mem. 108, 136–144. https://doi.org/10.1016/J.NLM.2013.11.019

Gazzano, A., Mariti, C., Notari, L., Sighieri, C., McBride, E.A., 2008. Effects of early gentling and early environment on emotional development of puppies. Appl. Anim. Behav. Sci. 110, 294–304. https://doi.org/10.1016/j.applanim.2007.05.007

Gensini, G., Yacoub, M., Conti, A., 2004. The concept of quarantine in history: from plague to SARS. J. Infect. 49, 257–261.

Gieling, E.T., Nordquist, R.E., van der Staay, F.J., 2011. Assessing learning and memory in pigs. Anim. Cogn. 14, 151–173. https://doi.org/10.1007/s10071-010-0364-3

Gimpl, G., Fahrenholz, F., 2001. The oxytocin receptor system: structure, function and regulation. Physiological reviews 81, 629-683.

Glickman, L.T., Shofer, F.S., 1987. Zoonotic Visceral and Ocular Larva Migrans. Vet. Clin. North Am. Small Anim. Pract. 17, 39–53. https://doi.org/10.1016/S0195-5616(87)50604-0

Goddard, M.E., Beilharz, R.G., 1983. Genetics of traits which determine the suitability of dogs as guide-dogs for the blind. Appl. Anim. Ethol. 9, 299–315. https://doi.org/10.1016/0304-3762(83)90010-X

Goddard, M.E., Beilharz, R.G., 1982. Genetic and environmental factors affecting the suitability of dogs as Guide Dogs for the Blind. Theor. Appl. Genet. 62, 97–102. https://doi.org/10.1007/BF00293339

Goldstein, O., Zangerl, B., Pearce-Kelling, S., Sidjanin, D.J., Kijas, J.W., Felix, J., Acland, G.M., Aguirre, G.D., 2006. Linkage disequilibrium mapping in domestic dog breeds narrows the progressive rod–cone degeneration interval and identifies ancestral disease-transmitting chromosome. Genomics 88, 541–550. https://doi.org/10.1016/J.YGENO.2006.05.013

Gordon, J.C., Angrick, E.J., 1986. Canine parvovirus: environmental effects on infectivity. Am. J. Vet. Res. 47, 1464–7.

Gortel, K., 2006. Update on canine demodicosis. Vet. Clin. North Am. Small Anim. Pract. 36, 229–41, ix. https://doi.org/10.1016/j.cvsm.2005.09.003

Gray, R., Butler, S., Douglas, C., Serpell, J., 2016. Puppies from “puppy farms” show more temperament and behavioural problems than if acquired from other sources, in: UFAW Animal Welfare Conference. Newcastle University, York, UK.

Gross, B., Garcia-Tapia, D., Riedesel, E., Ellinwood, N.M., Jens, J.K., 2010. NORMAL CANINE BRAIN MATURATION AT MAGNETIC RESONANCE IMAGING. Vet. Radiol. Ultrasound 51, 361–373. https://doi.org/10.1111/j.1740-8261.2010.01681.x

Guintard, C., Class, A.M., 2017. Hypertypes et standards de races chez le chien: Une histoire d’équilibre. Bull. Acad. Vet. Fr. 170, 230–248. https://doi.org/10.4267/2042/67199

Hamid, A.A., Pettibone, J.R., Mabrouk, O.S., Hetrick, V.L., Schmidt, R., Vander Weele, C.M., Kennedy, R.T., Aragona, B.J., Berke, J.D., 2016. Mesolimbic dopamine signals the value of work. Nat. Neurosci. 19, 117–126. https://doi.org/10.1038/nn.4173

Hare, B., Brown, M., Williamson, C., Tomasello, M., 2002. The domestication of social cognition in dogs. Science 298, 1634–6. https://doi.org/10.1126/science.1072702

HAS Kennistransfer & Bedrijfsopleidingen, 2015. Feiten & Cijfers Gezelschapsdierensector 2015, HAS Hogeschool & Utrecht University faculty of Veterinary medicine. Den Bosch.

Healey, M.C., Gaafar, S.M., 1977. Immunodeficiency in canine demodectic mange. I. Experimental production of lesions using antilymphocyte serum. Vet. Parasitol. 3, 121–131. https://doi.org/10.1016/0304-4017(77)90029-2

Hird, D., Ruble, R., Reagor, Sg.D.D. et al 1992. pdfFARE/Hir. et al 1992. pd., 1992. Morbidity and mortality in pups from pet stores and private sources: 968 cases (1987-1988). J. Am. Vet. Med. Assoc. 201, 471–474.

Horn, L., Huber, L., Range, F., 2013. The Importance of the Secure Base Effect for Domestic Dogs - Evidence from a Manipulative Problem-Solving Task. PLoS One 8, e65296. https://doi.org/10.1371/journal.pone.0065296

Horváth, Z., Igyártó, B.-Z., Magyar, A., Miklósi, Á., 2007. Three different coping styles in police dogs exposed to a short-term challenge. Horm. Behav. 52, 621–630. https://doi.org/10.1016/J.YHBEH.2007.08.001

Hsu, Y., Sun, L., 2010. Factors associated with aggressive responses in pet dogs. Appl. Anim. Behav. Sci. 123, 108–123. https://doi.org/10.1016/J.APPLANIM.2010.01.013

Hubrecht, R.C., 1995. Enrichment in puppyhood and its effects on later behavior of dogs. Lab. Anim. Sci. 45, 70–5.

Hubrecht, R.C., 1993. A comparison of social and environmental enrichment methods for laboratory housed dogs. Appl. Anim. Behav. Sci. 37, 345–361. https://doi.org/10.1016/0168-1591(93)90123-7

Hurley, K., 2009. Outbreak Management, in: Miller, L., Hurley, K. (Eds.), Infectious Disease Management in Animal Shelters. Wiley-Blackwell, p. 384.

Husemann, M., Zachos, F.E., Paxton, R.J., Habel, J.C., 2016. Effective population size in ecology and evolution. Heredity 117, 191-192.

Immelmann, K., Suomi, S.J., 1981. Sensitive phases in development, Behavioral Development. Cambridge University Press.

Insel, T.R., 2010. The Challenge of Translation in Social Neuroscience: A Review of Oxytocin, Vasopressin, and Affiliative Behavior. Neuron 65, 768–779. https://doi.org/10.1016/J.NEURON.2010.03.005

Itoh, N., Itagaki, T., Kawabata, T., Konaka, T., Muraoka, N., Saeki, H., Kanai, K., Chikazawa, S., Hori, Y., Hoshi, F., Higuchi, S., 2011. Prevalence of intestinal parasites and genotyping of Giardia intestinalis in pet shop puppies in east Japan, Veterinary Parasitology. https://doi.org/10.1016/j.vetpar.2010.10.048

Itoh, N., Kanai, K., Hori, Y., Hoshi, F., Higuchi, S., 2009. Prevalence of Giardia intestinalis and other zoonotic intestinal parasites in private household dogs of the Hachinohe area in

Aomori prefecture, Japan in 1997, 2002 and 2007. J. Vet. Sci. 10, 305. https://doi.org/10.4142/jvs.2009.10.4.305

Itoh, N., Muraoka, N., Saeki, H., Aoki, M., Itagaki, T., 2005. Prevalence of Giardia intestinalis Infection in Dogs of Breeding Kennels in Japan. J. Vet. Med. Sci. 67, 717–718. https://doi.org/10.1292/jvms.67.717

Izquierdo, I., Medina, J.H., 1997. Memory Formation: The Sequence of Biochemical Events in the Hippocampus and Its Connection to Activity in Other Brain Structures. Neurobiol. Learn. Mem. 68, 285–316. https://doi.org/10.1006/NLME.1997.3799

Jansen, A., Schöneberg, I., Frank, C., Alpers, K., Schneider, T., Stark, K., 2005. Leptospirosis in Germany, 1962-2003. Emerg. Infect. Dis. 11, 1048–54. https://doi.org/10.3201/eid1107.041172

Jokinen, O., Appleby, D., Sandbacka-Saxén, S., Appleby, T., Valros, A., 2017. Homing age influences the prevalence of aggressive and avoidance-related behaviour in adult dogs. Appl. Anim. Behav. Sci. 195, 87–92. https://doi.org/10.1016/J.APPLANIM.2017.06.003

Kapel, C.M.O., Torgerson, P.R., Thompson, R.C.A., Deplazes, P., 2006. Reproductive potential of Echinococcus multilocularis in experimentally infected foxes, dogs, raccoon dogs and cats. Int. J. Parasitol. 36, 79–86. https://doi.org/10.1016/j.ijpara.2005.08.012

Kendrick, K.M., 2000. Oxytocin, motherhood and bonding. Exp. Physiol. 85, 111s–124s. https://doi.org/10.1111/j.1469-445X.2000.tb00014.x

KennelClub, 2018. History of The Kennel Club [WWW Document]. URL https://www.thekennelclub.org.uk/our-resources/about-the-kennel-club/history-of-the-kennel-club/

Kerswell, K.J., Butler, K.L., Bennett, P., Hemsworth, P.H., 2010. The relationships between morphological features and social signalling behaviours in juvenile dogs: The effect of early experience with dogs of different morphotypes. Behav. Processes 85, 1–7. https://doi.org/10.1016/J.BEPROC.2010.05.006

King, T., Marston, L.C., Bennett, P.C., 2012. Breeding dogs for beauty and behaviour: Why scientists need to do more to develop valid and reliable behaviour assessments for dogs kept as companions. Appl. Anim. Behav. Sci. 137, 1–12. https://doi.org/10.1016/J.APPLANIM.2011.11.016

Kirkpatrick, C.E., 1988. Epizootiology of endoparasitic infections in pet dogs and cats presented to a veterinary teaching hospital. Vet. Parasitol. 30, 113–124. https://doi.org/10.1016/0304-4017(88)90158-6

Kis, A., Hernádi, A., Kanizsár, O., Gácsi, M., Topál, J., 2015. Oxytocin induces positive expectations about ambivalent stimuli (cognitive bias) in dogs. Horm. Behav. 69, 1–7. https://doi.org/10.1016/J.YHBEH.2014.12.004

Klein, S.L., Rager, D.R., 1995. Prenatal stress alters immune function in the offspring of rats. Dev. Psychobiol. 28, 321–336. https://doi.org/10.1002/dev.420280603

Koolhaas, J.., Korte, S.., De Boer, S.., Van Der Vegt, B.., Van Reenen, C.., Hopster, H., De Jong, I.., Ruis, M.A.., Blokhuis, H.., 1999. Coping styles in animals: current status in behavior and stress-physiology. Neurosci. Biobehav. Rev. 23, 925–935. https://doi.org/10.1016/S0149-7634(99)00026-3

Koolhaas, J.M., de Boer, S.F., Coppens, C.M., Buwalda, B., 2010. Neuroendocrinology of coping styles: Towards understanding the biology of individual variation. Front. Neuroendocrinol. 31, 307–321. https://doi.org/10.1016/j.yfrne.2010.04.001

Korbelik, J., Rand, J.S., Morton, J.M., 2011. Comparison of early socialization practices used for litters of small-scale registered dog breeders and nonregistered dog breeders. J. Am. Vet. Med. Assoc. 239, 1090–1097. https://doi.org/10.2460/javma.239.8.1090

Kumsta, R., Hummel, E., Chen, F.S., Heinrichs, M., 2013. Epigenetic regulation of the oxytocin receptor gene: implications for behavioral neuroscience. Front. Neurosci. 7, 83. https://doi.org/10.3389/fnins.2013.00083

Landsberg, G., 2001. Chapter 2 - Behavior Development and Preventive Management, in: Hoskins, J.D. (Ed.), Veterinary Pediatrics (Third Edition). W.B. Saunders, Philadelphia, pp. 22–34. https://doi.org/http://dx.doi.org/10.1016/B978-0-7216-7665-4.50006-1

Larson, G., Karlsson, E.K., Perri, A., Webster, M.T., Ho, S.Y.W., Peters, J., Stahl, P.W., Piper, P.J., Lingaas, F., Fredholm, M., Comstock, K.E., Modiano, J.F., Schelling, C., Agoulnik, A.I., Leegwater, P.A., Dobney, K., Vigne, J.-D., Vila, C., Andersson, L., Lindblad-Toh, K., 2012. Rethinking dog domestication by integrating genetics, archeology, and biogeography. Proc. Natl. Acad. Sci. 109, 8878–8883. https://doi.org/10.1073/pnas.1203005109

Lee, J.-H., Kim, H.J., Kim, J.G., Ryu, V., Kim, B.-T., Kang, D.-W., Jahng, J.W., 2007. Depressive behaviors and decreased expression of serotonin reuptake transporter in rats that experienced neonatal maternal separation. Neurosci. Res. 58, 32–39. https://doi.org/10.1016/J.NEURES.2007.01.008

Lee, P.R., Brady, D.L., Shapiro, R.A., Dorsa, D.M., Koenig, J.I., 2007. Prenatal stress generates deficits in rat social behavior: Reversal by oxytocin. Brain Res. 1156, 152–167. https://doi.org/10.1016/j.brainres.2007.04.042

Leroy, G., 2011. Genetic diversity, inbreeding and breeding practices in dogs: Results from pedigree analyses. Vet. J. 189, 177–182. https://doi.org/10.1016/j.tvjl.2011.06.016

Leroy, H., Depiereux, E., Giffroy, J.-M., Diederich, C., 2009. Effect of prenatal environment on learning abilities in puppies and in adult dogs. J. Vet. Behav. 4, 253. https://doi.org/10.1016/j.jveb.2009.06.008

Lillard, A.S., Erisir, A., 2011. Old dogs learning new tricks: Neuroplasticity beyond the juvenile period. Dev. Rev. 31, 207–239. https://doi.org/10.1016/J.DR.2011.07.008

Lindblad-Toh, K., Wade, C.M., Mikkelsen, T.S., Karlsson, E.K., Jaffe, D.B., Kamal, M., Clamp, M., Chang, J.L., Kulbokas, E.J., Zody, M.C., 2005. Genome sequence, comparative analysis and haplotype structure of the domestic dog. Nature 438, 803–819. https://doi.org/10.1038/nature04338

Liu, D., Diorio, J., Tannenbaum, B., Caldji, C., Francis, D., Freedman, A., Sharma, S., Pearson, D., Plotsky, P.M., Meaney, M.J., 1997. Maternal Care, Hippocampal Glucocorticoid Receptors, and Hypothalamic-Pituitary-Adrenal Responses to Stress. Science (80-.). 277, 1659–1662.

Martella, V., Elia, G., Buonavoglia, C., 2008. Canine distemper virus. Vet Clin North Am Small Anim Pr. 38, 787–97, vii–viii. https://doi.org/10.1016/j.cvsm.2008.02.007

Martínez, Á.G., Santamarina Pernas, G., Diéguez Casalta, F.J., Suárez Rey, M.L., De la Cruz Palomino, L.F., 2011. Risk factors associated with behavioral problems in dogs. J. Vet. Behav. Clin. Appl. Res. 6, 225–231. https://doi.org/10.1016/j.jveb.2011.01.006

Mason, J., Arzt, A., Auk, R.R.-T., 1984, U., 1984. Comparative assessment of food preferences and aversions acquired by blackbirds via observational learning. Auk - Ornithol. Adv. 101.

McCall, C., Singer, T., 2012. The animal and human neuroendocrinology of social cognition, motivation and behavior. Nat. Neurosci. 15, 681–688. https://doi.org/10.1038/nn.3084

McDonnell, G., Russell, A.D., 1999. Antiseptics and disinfectants: activity, action, and resistance. Clin. Microbiol. Rev. 12, 147–79.

McEwen, B.S., Sapolsky, R.M., 1995. Stress and cognitive function. Curr. Opin. Neurobiol. 5, 205–216. https://doi.org/10.1016/0959-4388(95)80028-X

McGowan, R.T.S.S., Rehn, T., Norling, Y., Keeling, L.J.L.J., 2014. Positive affect and learning: exploring the “Eureka Effect” in dogs. Anim. Cogn. 17, 577–587. https://doi.org/10.1007/s10071-013-0688-x

McGreevy, P.D., Georgevsky, D., Carrasco, J., Valenzuela, M., Duffy, D.L., Serpell, J.A., 2013. Dog Behavior Co-Varies with Height, Bodyweight and Skull Shape. PLoS One 8, e80529. https://doi.org/10.1371/journal.pone.0080529

McMillan, F.D., 2017. Behavioral and psychological outcomes for dogs sold as puppies through pet stores and/or born in commercial breeding establishments: Current knowledge and

putative causes. J. Vet. Behav. Clin. Appl. Res. 19, 14–26. https://doi.org/10.1016/j.jveb.2017.01.001

McMillan, F.D., Serpell, J.A., Duffy, D.L., Masaoud, E., Dohoo, I.R., 2013. Differences in behavioral characteristics between dogs obtained as puppies from pet stores and those obtained from noncommercial breeders. J. Am. Vet. Med. Assoc. 242, 1359–1363. https://doi.org/10.2460/javma.242.10.1359

Meaney, M.J., 2001. Maternal Care, Gene Expression, and the Transmission of Individual Differences in Stress Reactivity Across Generations. Annu. Rev. Neurosci. 24, 1161–1192. https://doi.org/10.1146/annurev.neuro.24.1.1161

Merola, I., Prato-Previde, E., Marshall-Pescini, S., 2012a. Social referencing in dog-owner dyads? Anim. Cogn. 15, 175–185. https://doi.org/10.1007/s10071-011-0443-0

Merola, I., Prato-Previde, E., Marshall-Pescini, S., 2012b. Dogs' Social Referencing towards Owners and Strangers. PLoS One 7, e47653. https://doi.org/10.1371/journal.pone.0047653

Meunier, L.D., 2006. Selection, Acclimation, Training, and Preparation of Dogs for the Research Setting. ILAR J. 47, 326–347. https://doi.org/10.1093/ilar.47.4.326

Miklósi, Á., Topál, J., 2013. What does it take to become 'best friends'? Evolutionary changes in canine social competence. Trends Cogn. Sci. 17, 287–294. https://doi.org/10.1016/j.tics.2013.04.005

Miller, L., Zawistowski, S., 2013. Shelter medicine for veterinarians and staff. Wiley-Blackwell.

Miranda, C., Thompson, G., 2016. Canine parvovirus: the worldwide occurrence of antigenic variants. J. Gen. Virol. 97, 2043–2057. https://doi.org/10.1099/jgv.0.000540

Mircean, V., Györke, A., Cozma, V., 2012. Prevalence and risk factors of Giardia duodenalis in dogs from Romania, Veterinary Parasitology. https://doi.org/10.1016/j.vetpar.2011.08.022

Mortel, T. van de, 2008. Faking it: social desirability response bias in self-report research. Aust. J. Adv. Nurs. 25, 40–48.

Münnich, A., Küchenmeister, U., 2014. Causes, Diagnosis and Therapy of Common Diseases in Neonatal Puppies in the First Days of Life: Cornerstones of Practical Approach. Reprod. Domest. Anim. 49, 64–74. https://doi.org/10.1111/rda.12329

Münnich, A., Lübke-Becker, A., 2004. Escherichia coli infections in newborn puppies—clinical and epidemiological investigations. Theriogenology. 62, 562-75. https://doi.org/10.1016/j.theriogenology.2003.11.012

Nagasawa, M., Kikusui, T., Onaka, T., Ohta, M., 2009. Dog's gaze at its owner increases owner's urinary oxytocin during social interaction. Horm. Behav. 55, 434–441. https://doi.org/https://doi.org/10.1016/j.yhbeh.2008.12.002

Nagasawa, M., Okabe, S., Mogi, K., Kikusui, T., 2012. Oxytocin and mutual communication in mother-infant bonding. Front. Hum. Neurosci. 6, 1–10. https://doi.org/10.3389/fnhum.2012.00031

Nagasawa, M., Shibata, Y., Yonezawa, A., Morita, T., Kanai, M., Mogi, K., Kikusui, T., 2014. The behavioral and endocrinological development of stress response in dogs. Dev. Psychobiol. 56, 726–733. https://doi.org/10.1002/dev.21141

Naylor, M., Monckton, R., Lehrbach, P., 2001. Canine coronavirus in Australian dogs. Aust. Vet. J. 79, 116–119. https://doi.org/10.1111/j.1751-0813.2001.tb10718.x

Nelson, R.J., Trainor, B.C., 2007. Neural mechanisms of aggression. Nat. Rev. Neurosci. 8, 536–546. https://doi.org/10.1038/nrn2174

Newbury, E., Miller, L., 2018. Biosecurity in shelters, in: BSAVA Manual of Canine and Feline Shelter Medicine. British Small Animal Veterinary Association, pp. 112–125. https://doi.org/10.22233/9781910443330.9

Nielen, A.L., van der Gaag, I., Knol, B.W., Schukken, Y.H., 1998. Investigation of mortality and pathological changes in a 14-month birth cohort of boxer puppies. Vet. Rec. 142, 602–6. https://doi.org/10.1136/VR.142.22.602

Olivier, J.D.A., Cools, A.R., Deen, P.M.T., Olivier, B., Ellenbroek, B.A., 2010. Blockade of dopamine, but not noradrenaline, transporters produces hyperthermia in rats that lack serotonin transporters. Eur. J. Pharmacol. 629, 7–11. https://doi.org/10.1016/J.EJPHAR.2009.11.049

Otranto, D., Milillo, P., Mesto, P., De Caprariis, D., Perrucci, S., Capelli, G., 2004. Otodectes cynotis (Acari: Psoroptidae): examination of survival off-the-host under natural and laboratory conditions. Exp. Appl. Acarol. 32, 171–180. https://doi.org/10.1023/B:APPA.0000021832.13640.ff

Overall, K.L., 2013. Manual of clinical behavioral medicine for dogs and cats. Elsevier Mosby Publishing, St. Louis.

Overgaauw, P.A., van Knapen, F., 2013. Veterinary and public health aspects of Toxocara spp. Vet Parasitol 193, 398–403. https://doi.org/10.1016/j.vetpar.2012.12.035

Overgaauw, P.A.M., Boersema, J.H., 1998. Nematode infections in dog breeding kennels in the Netherlands, with special reference to Toxocara. Vet. Q. 20, 12–15. https://doi.org/10.1080/01652176.1998.9694827

Parker, H.G., Kim, L. V, Sutter, N.B., Carlson, S., Lorentzen, T.D., Malek, T.B., Johnson, G.S., DeFrance, H.B., Ostrander, E.A., Kruglyak, L., 2004. Genetic Structure of the Purebred Domestic Dog. Science (80-.). 304, 1160 LP-1164. https://doi.org/10.1126/science.1097406

Patel, J.R., Heldens, J.G.M., 2009. Review of companion animal viral diseases and immunoprophylaxis. Vaccine 27, 491–504. https://doi.org/http://dx.doi.org/10.1016/j.vaccine.2008.11.027

Patronek, G.J., Glickman, L.T., Beck, A.M., McCabe, G.P., Ecker, C., 1996. Risk factors for relinquishment of dogs to an animal shelter. J. Am. Vet. Med. Assoc. 209, 572–81.

Pavlov, P.I., 1927. Conditioned reflexes: An investigation of the physiological activity of the cerebral cortex. Ann. Neurosci. 17, 136–41. https://doi.org/10.5214/ans.0972-7531.1017309

Payne, E., Bennett, P., McGreevy, P., 2015. Current perspectives on attachment and bonding in the dog–human dyad. Psychol. Res. Behav. Manag. 71. https://doi.org/10.2147/PRBM.S74972

Penza, K.M., Heim, C., Nemeroff, C.B., 2003. Neurobiological effects of childhood abuse: implications for the pathophysiology of depression and anxiety. Arch. Womens. Ment. Health 6, 15–22. https://doi.org/10.1007/s00737-002-0159-x

Piaget, J., 1977. The development of thought : equilibration of cognitive structures. Viking Press, New York.

Pierantoni, L., Albertini, M., Pirrone, F., 2011. Prevalence of owner-reported behaviours in dogs separated from the litter at two different ages. Vet. Rec. 169. https://doi.org/10.1136/vr.d4967

Pratelli, A., 2006. Genetic evolution of canine coronavirus and recent advances in prophylaxis. Vet. Res. 37, 191–200. https://doi.org/10.1051/vetres:2005053

Pratelli, A., Martella, V., Elia, G., Tempesta, M., Guarda, F., Capucchio, M.T., Carmichael, L.E., Buonavoglia, C., 2001. Severe Enteric Disease in an Animal Shelter Associated with Dual Infections by Canine Adenovirus Type 1 and Canine Coronavirus. J. Vet. Med. Ser. B 48, 385–392. https://doi.org/10.1046/j.1439-0450.2001.00466.x

Previde, E.P., Ghirardelli, G., Marshall-Pescini, S., Valsecchi, P., 2009. Intraspecific attachment in domestic puppies (Canis familiaris). J. Vet. Behav. Clin. Appl. Res. 4, 89–90. https://doi.org/10.1016/J.JVEB.2008.10.002

Protopopova, A., 2016. Effects of sheltering on physiology, immune function, behavior, and the welfare of dogs. Physiol. Behav. 159, 95–103.

Radek, K.A., 2010. Antimicrobial anxiety: the impact of stress on antimicrobial immunity. J. Leukoc. Biol. 88, 263–77. https://doi.org/10.1189/jlb.1109740

Ramírez-Barrios, R.A., Barboza-Mena, G., Muñoz, J., Angulo-Cubillán, F., Hernández, E., González, F., Escalona, F., 2004. Prevalence of intestinal parasites in dogs under veterinary care in Maracaibo, Venezuela. Vet. Parasitol. 121, 11–20. https://doi.org/10.1016/J.VETPAR.2004.02.024

Range, F., Viranyi, Z., Huber, L., 2007. Selective Imitation in Domestic Dogs. Curr. Biol. 17, 868–872. https://doi.org/10.1016/J.CUB.2007.04.026

Rankin, C.H., Abrams, T., Barry, R.J., Bhatnagar, S., Clayton, D., Colombo, J., Coppola, G., Geyer, M.A., Glanzman, D.L., Marsland, S., Mcsweeney, F., Wilson, D.A., Wu, C., Thompson, R.F., 2009. Habituation Revisited: An Updated and Revised Description of the Behavioral Characteristics of Habituation Catharine. Neurobiol. Learn. Mem. 92, 135–138. https://doi.org/10.1016/j.nlm.2008.09.012.Habituation

Riemer, S., Müller, C., Virányi, Z., Huber, L., Range, F., 2013. Choice of conflict resolution strategy is linked to sociability in dog puppies. Appl. Anim. Behav. Sci. 149, 36–44. https://doi.org/10.1016/j.applanim.2013.09.006

Rimmelzwaan, G.F., Groen, J., Egberink, H., Borst, G.H.A., UytdeHaag, F.G.C.M., Osterhaus, A.D.M.E., 1991. The use of enzyme-linked immunosorbent assay systems for serology and antigen detection in parvovirus, coronavirus and rotavirus infections in dogs in The Netherlands. Vet. Microbiol. 26, 25–40. https://doi.org/10.1016/0378-1135(91)90039-I

Robertson, I.D., Irwin, P.J., Lymbery, A.J., Thompson, R.C.A., 2000. The role of companion animals in the emergence of parasitic zoonoses. Int. J. Parasitol. 30, 1369–1377. https://doi.org/10.1016/S0020-7519(00)00134-X

Robinson, W.H., 1995. Distribution of Cat Flea Larvae in the Carpeted Household Environment. Vet. Dermatol. 6, 145–150. https://doi.org/10.1111/j.1365-3164.1995.tb00058.x

Romero, T., Nagasawa, M., Mogi, K., Hasegawa, T., Kikusui, T., 2014. Oxytocin promotes social bonding in dogs. Proc. Natl. Acad. Sci. U. S. A. 111, 9085–90. https://doi.org/10.1073/pnas.1322868111

Ronsse, V., Verstegen, J., Onclin, K., Guiot, A., Aeberle, C., Nauwynck, H., Poulet, H., 2002. Seroprevalence of Canine Herpesvirus-1 in the Belgian Dog Population in 2000. Reprod. Domest. Anim. 37, 299–304. https://doi.org/10.1046/j.1439-0531.2002.00363.x

Ronsse, V., Verstegen, J., Thiry, E., Onclin, K., Aeberle, C., Brunet, S., Poulet, H., 2005. Canine herpesvirus-1 (CHV-1): clinical, serological and virological patterns in breeding colonies. Theriogenology 64, 61–74. https://doi.org/10.1016/j.theriogenology.2004.11.016

Rooney, N.J., 2009. The welfare of pedigree dogs: Cause for concern. J. Vet. Behav. 4, 180–186. https://doi.org/10.1016/J.JVEB.2009.06.002

Root Kustritz, M. V, 2011. Chapter 4 - History and Physical Examination of the Weanling and Adolescent, in: Kutzler, M.E.P.A. (Ed.), Small Animal Pediatrics. W.B. Saunders, Saint Louis, pp. 28–33. https://doi.org/http://dx.doi.org/10.1016/B978-1-4160-4889-3.00004-8

Sachser, N., Kaiser, S., 1996. Prenatal Social Stress Masculinizes the Females' Behaviour in Guinea Pigs, Physiology & Behavior.

Salib, F.A., Baraka, T.A., 2011. Epidemiology, genetic divergence and acaricides for Otodectes cynotis in cats and dogs. Vet. World 4, 109–112.

Sampson, J., Binns, M.M., 2006. The Kennel Club and the early history of dog shows and breed clubs, in: Ostrander, E.A., Giger, U., Lindblad-Toh, K. (Eds.), The Dog and Its Genome. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 19–30. https://doi.org/10.1101/087969781.44.19

Sapolsky, R.M., Romero, L.M., Munck, A.U., 2000. How Do Glucocorticoids Influence Stress Responses? Integrating Permissive, Suppressive, Stimulatory, and Preparative Actions 1. Endocr. Rev. 21, 55–89. https://doi.org/10.1210/edrv.21.1.0389

Sarnyai, Z., Kovács, G.L., 2014. Oxytocin in learning and addiction: From early discoveries to the present. Pharmacol. Biochem. Behav. 119, 3–9. https://doi.org/10.1016/J.PBB.2013.11.019

Sarrazin, S., Cay, A.B., Laureyns, J., Dewulf, J., 2014. A survey on biosecurity and management practices in selected Belgian cattle farms. Prev. Vet. Med. 117, 129–139. https://doi.org/10.1016/j.prevetmed.2014.07.014

Schäfer-Somi, S., Spergser, J., Breitenfellner, J., Aurich, J.E., 2003. Bacteriological status of canine milk and septicaemia in neonatal puppies--a retrospective study. J. Vet. Med. B. Infect. Dis. Vet. Public Health 50, 343–6.

Scholthof, K.-B.G., 2007. The disease triangle: pathogens, the environment and society. Nat. Rev. Microbiol. 5, 152–156. https://doi.org/10.1038/nrmicro1596

Schrijver, R., Sikkema, R., de Vries, H., Dewar, D., Bergevoet, R., Messori, S., D'Albenzio, S., Barnard, S., 2015. Study on the welfare of dogs and cats involved in commercial practices. https://ec.europa.eu/food/sites/food/files/animals/docs/aw_eu-strategy_study_dogs-cats-commercial-practices_en.pdf

Schulz, B.S., Strauch, C., Mueller, R.S., Eichhorn, W., Hartmann, K., 2008. Comparison of the prevalence of enteric viruses in healthy dogs and those with acute haemorrhagic diarrhoea by electron microscopy. J. Small Anim. Pract. 49, 84–88. https://doi.org/10.1111/j.1748-5827.2007.00470.x

Schumaker, B.A., Miller, M.M., Grosdidier, P., Cavender, J.L., Montgomery, D.L., Cornish, T.E., Farr, R.M., Driscoll, M., Maness, L.J., Gray, T., Petersen, D., Brown, W.L., Logan, J., O'Toole, D., 2012. Canine distemper outbreak in pet store puppies linked to a high-volume dog breeder. J. Vet. Diagnostic Investig. 24, 1094–1098. https://doi.org/10.1177/1040638712460531

Scott, A., Zepeda, C., Garber, L., Smith, J., Swayne, D., Rhorer, A., Giovannini, A., 2006. The concept of compartmentalisation. Rev. Sci. Tech. Int. des Epizoot. 25.

Scott, J.P., Fuller, J.L., 1965. Genetics and the Social Behavior of the Dog. University of Chicago Press, Chicago.

Seksel, K., Mazurski, E.E.J., Taylor, A., 1999. Puppy socialisation programs: short and long term behavioural effects. Appl. Anim. Behav. Sci. 62, 335–349. https://doi.org/http://dx.doi.org/10.1016/S0168-1591(98)00232-9

Serpell, J.A., Duffy, D.L., Serpell, J.A., Duffy, D.L., 2014. Dog Breeds and Their Behavior, Domestic Dog Cognition and Behavior. Springer Berlin Heidelberg, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-53994-7_2

Silva, F., Oliveira, R., Silva, T., Xavier, M., 2009. Venereal transmission of canine visceral leishmaniasis. Vet. Parasitol. 160, 55-59. https://doi.org/10.1016/j.vetpar.2008.10.079

Skinner, B.F., Burrhus F., 1953. Science and human behavior. Macmillan.

Slabbert, J.M., Rasa, O.A., 1993. The effect of early separation from the mother on pups in bonding to humans and pup health. J. S. Afr. Vet. Assoc. 64, 4–8.

Sobrino, R., Arnal, M.C., Luco, D.F., Gortázar, C., 2008. Prevalence of antibodies against canine distemper virus and canine parvovirus among foxes and wolves from Spain. Vet. Microbiol. 126, 251–256. https://doi.org/10.1016/J.VETMIC.2007.06.014

Song, D., Kang, B., Lee, C., Jung, K., Ha, G., Kang, D., Park, S., Park, B., Oh, J., 2008. Transmission of avian influenza virus (H3N2) to dogs. Emerg. Infect. Dis. 14, 741–6. https://doi.org/10.3201/eid1405.071471

Spady, T.C., Ostrander, E.A., 2008. Canine Behavioral Genetics: Pointing Out the Phenotypes and Herding up the Genes. Am. J. Hum. Genet. 82, 10–18. https://doi.org/10.1016/J.AJHG.2007.12.001

STATBEL, 2010. De honden en katten van de Belgen [WWW Document]. URL http://statbel.fgov.be/nl/binaries/De honden en katten van de Belgen_2008_nl_tcm325-103755.pdf

Stavisky, J., Pinchbeck, G., Gaskell, R.M., Dawson, S., German, A.J., Radford, A.D., 2012. Cross sectional and longitudinal surveys of canine enteric coronavirus infection in

kennelled dogs: A molecular marker for biosecurity. Infect. Genet. Evol. 12, 1419–1426. https://doi.org/10.1016/j.meegid.2012.04.010

Stavisky, J., Pinchbeck, G.L., German, A.J., Dawson, S., Gaskell, R.M., Ryvar, R., Radford, A.D., 2010. Prevalence of canine enteric coronavirus in a cross-sectional survey of dogs presenting at veterinary practices. Vet. Microbiol. 140, 18–24. https://doi.org/10.1016/j.vetmic.2009.07.012

Stehr-Green, J., Murray, G., 1987. Intestinal parasites in pet store puppies in Atlanta. Am. J. Public Health 77, 345–346.

Stepita, M.E., Bain, M.J., Kass, P.H., 2013. Frequency of CPV Infection in Vaccinated Puppies that Attended Puppy Socialization Classes. J. Am. Anim. Hosp. Assoc. 49, 95–100. https://doi.org/10.5326/JAAHA-MS-5825

Stoffel, M.H., Friess, A.E., Hartmann, S.H., 2000. Ultrastructural evidence of transplacental transport of immunoglobulin G in bitches. J. Reprod. Fertil. 118, 315–26.

Strandberg, E., Jacobsson, J., Saetre, P., 2005. Direct genetic, maternal and litter effects on behaviour in German shepherd dogs in Sweden. Livest. Prod. Sci. 93, 33–42. https://doi.org/10.1016/j.livprodsci.2004.11.004

Stull, J.W., Kasten, J.I., Evason, M.D., Sherding, R.G., Hoet, A.E., O'Quin, J., Burkhard, M.J., Weese, J.S., 2016. Risk reduction and management strategies to prevent transmission of infectious disease among dogs at dog shows, sporting events, and other canine group settings. J. Am. Vet. Med. Assoc. 249, 612–627. https://doi.org/10.2460/javma.249.6.612

Sullivan, R., Wilson, D., Leon, M., 1989. Norepinephrine and learning-induced plasticity in infant rat olfactory system. J. Neurosci. 9, 3998–4006. https://doi.org/10.1523/JNEUROSCI.09-11-03998.1989

Sutter, N.B., Ostrander, E.A., 2004. Dog star rising: the canine genetic system. Nat. Rev. Genet. 5, 900–910.

Svartberg, K., 2002. Shyness–boldness predicts performance in working dogs. Appl. Anim. Behav. Sci. 79, 157–174. https://doi.org/10.1016/S0168-1591(02)00120-X

Thalmann, O., Shapiro, B., Cui, P., Schuenemann, V.J., Sawyer, S.K., Greenfield, D.L., Germonpré, M.B., Sablin, M. V., López-Giráldez, F., Domingo-Roura, X., Napierala, H., Uerpmann, H.P., Loponte, D.M., Acosta, A.A., Giemsch, L., Schmitz, R.W., Worthington, B., Buikstra, J.E., Druzhkova, A., Graphodatsky, A.S., Ovodov, N.D., Wahlberg, N., Freedman, A.H., Schweizer, R.M., Koepfli, K.P., Leonard, J.A., Meyer, M., Krause, J., Pääbo, S., Green, R.E., Wayne, R.K., 2013. Complete mitochondrial genomes of ancient canids suggest a European origin of domestic dogs. Science 342, 871–874. https://doi.org/10.1126/science.1243650

Thompson, R.F., Spencer, W.A., 1966. Habituation: A model phenomenon for the study of neuronal substrates of behavior. Psychol. Rev. 73, 16–43. https://doi.org/10.1037/h0022681

Thorndike, E.L., 1927. The Law of Effect. Am. J. Psychol. 39, 212. https://doi.org/10.2307/1415413

Tiira, K., Lohi, H., 2015. Early Life Experiences and Exercise Associate with Canine Anxieties. PLoS One 10, e0141907. https://doi.org/10.1371/journal.pone.0141907

Toman, M., Faldyna, M., Knotigova, P., Pokorova, D., Sinkora, J., 2002. Postnatal development of leukocyte subset composition and activity in dogs. Vet. Immunol. Immunopathol. 87, 321–326. https://doi.org/10.1016/S0165-2427(02)00058-2

Topál, J., Gácsi, M., Miklósi, Á., Virányi, Z., Kubinyi, E., Csányi, V., 2005. Attachment to humans: a comparative study on hand-reared wolves and differently socialized dog puppies. Anim. Behav. 70, 1367–1375. https://doi.org/10.1016/j.anbehav.2005.03.025

Truyen, U., 1999. Emergence and recent evolution of canine parvovirus. Vet. Microbiol. 69, 47–50. https://doi.org/10.1016/S0378-1135(99)00086-3

Tsai, Y.-J., Chung, W.-C., Wang, L.-C., Yu-Ten, J., Hong, C.-L., Tsai, Y.-Y., Li, Y.-H., Wu, Y.-L., 2011. The dog mite, Demodex canis: Prevalence, fungal co-infection, reactions to light, and hair follicle apoptosis. J. Insect Sci. 11. https://doi.org/10.1673/031.011.7601

Tuchscherer, M., Kanitz, E., Otten, W., Tuchscherer, A., 2002. Effects of prenatal stress on cellular and humoral immune responses in neonatal pigs. Vet. Immunol. Immunopathol. 86, 195–203. https://doi.org/10.1016/S0165-2427(02)00035-1

U.S. District Court for the District of Minnesota, 1984. Avenson v. Zegart, 577 F. Supp. 958 (D. Minn. 1984) :: Justia [WWW Document]. URL https://law.justia.com/cases/federal/district-courts/FSupp/577/958/1497077/ (accessed 1.16.18).

van Rooy, D., Arnott, E.R., Early, J.B., McGreevy, P., Wade, C.M., 2014. Holding back the genes: limitations of research into canine behavioural genetics. Canine Genet. Epidemiol. 1, 1–11. https://doi.org/10.1186/2052-6687-1-7

Van Soom, A., Peelman, L, Holt, W.V., Fazeli, A., 2014. An Introduction to Epigenetics as the Link Between Genotype and Environment: A Personal View. Reproduction in Domestic Animals 49, 2-10.

Villarroel, A., Dargatz, D.A., Lane, V.M., McCluskey, B.J., Salman, M.D., 2007. Suggested outline of potential critical control points for biosecurity and biocontainment on large dairy farms. J. Am. Vet. Med. Assoc. 230, 808–819. https://doi.org/10.2460/javma.230.6.808

Vlaamse overheid dept. L&V, 2013. De economische waarde van de huisdierensector. https://lv.vlaanderen.be/sites/default/files/attachments/2013%2001%2021%20focusrapport%20huisdieren.pdf

vonHoldt, B.M., Pollinger, J.P., Lohmueller, K.E., Han, E., Parker, H.G., Quignon, P., Degenhardt, J.D., Boyko, A.R., Earl, D.A., Auton, A., Reynolds, A., Bryc, K., Brisbin, A., Knowles, J.C., Mosher, D.S., Spady, T.C., Elkahloun, A., Geffen, E., Pilot, M., Jedrzejewski, W., Greco, C., Randi, E., Bannasch, D., Wilton, A., Shearman, J., Musiani, M., Cargill, M., Jones, P.G., Qian, Z., Huang, W., Ding, Z.-L., Zhang, Y., Bustamante, C.D., Ostrander, E.A., Novembre, J., Wayne, R.K., 2010. Genome-wide SNP and haplotype analyses reveal a rich history underlying dog domestication. Nature 464, 898–902. https://doi.org/10.1038/nature08837

Wauthier, L.M., Scottish Society for the Prevention of Cruelty to Animals (Scottish SPCA), Williams, J.M., 2018. Using the mini C-BARQ to investigate the effects of puppy farming on dog behaviour. Appl. Anim. Behav. Sci. 206, 75–86. https://doi.org/10.1016/J.APPLANIM.2018.05.024

Welfare, B.C.A., 2014. Werkgroep: verhandeling van honden. https://www.lne.be/sites/default/files/atoms/files/Werkgroep%20Invoer%20van%20en%20handel%20in%20honden%20-%20Informatiefiche%20%282014%29.pdf

Wells, D.L., 2004. The influence of toys on the behaviour and welfare of kennelled dogs. Animal Welfare 13, 367-373.

Wilcox, B., Walkowicz, C., 1989. Atlas of dog breeds of the world. T.F.H. Publications.

Wilkerson, M.J., Bagladi-Swanson, M., Wheeler, D.W., Floyd-Hawkins, K., Craig, C., Lee, K.W., Dryden, M., 2004. The immunopathogenesis of flea allergy dermatitis in dogs, an experimental study. Vet. Immunol. Immunopathol. 99, 179–192. https://doi.org/10.1016/J.VETIMM.2004.02.006

Wilson, D.A., Sullivan, R.M., 1994. Neurobiology of associative learning in the neonate: Early olfactory learning. Behav. Neural Biol. 61, 1–18. https://doi.org/10.1016/S0163-1047(05)80039-1

Wilsson, E., 1984. The social interaction between mother and offspring during weaning in German shepherd dogs: individual differences between mothers 1. Wilsson E. 1984; The social interaction between mother and offspring during weaning in German shepherd dogs: individual d. Appl. Anim. Behav. Sci. 13, 101–112. https://doi.org/https://doi.org/10.1016/0168-1591(84)90056-X

Wilsson, E., Sundgren, P.-E., 1998a. Behaviour test for eight-week old puppies—heritabilities of tested behaviour traits and its correspondence to later behaviour. Appl. Anim. Behav. Sci. 58, 151–162. https://doi.org/http://dx.doi.org/10.1016/S0168-1591(97)00093-2

Wilsson, E., Sundgren, P.-E., 1998b. Effects of weight, litter size and parity of mother on the behaviour of the puppy and the adult dog. Appl. Anim. Behav. Sci. 56, 245–254. https://doi.org/https://doi.org/10.1016/S0168-1591(97)00094-4

Wobber, V., Hare, B., 2009. Testing the social dog hypothesis: Are dogs also more skilled than chimpanzees in non-communicative social tasks? Behav. Processes 81, 423–428. https://doi.org/10.1016/J.BEPROC.2009.04.003

Wyatt, T., Biddle, P., Maher, J., 2016. Sourcing of pet dogs from illegal importation and puppy farms 2016-2017: scoping research.

Young, L.J., Lim, M.M., Gingrich, B., Insel, T.R., 2001. Cellular Mechanisms of Social Attachment. Horm. Behav. 40, 133–138. https://doi.org/https://doi.org/10.1006/hbeh.2001.1691

Zhang, T.-Y., Meaney, M.J., 2010. Epigenetics and the Environmental Regulation of the Genome and Its Function. Annu. Rev. Psychol. 61, 439–466. https://doi.org/10.1146/annurev.psych.60.110707.163625

Zicola, A., Jolly, S., Mathijs, E., Ziant, D., Decaro, N., Mari, V., Thiry, E., 2012. Fatal outbreaks in dogs associated with pantropic canine coronavirus in France and Belgium. J. Small Anim. Pract. 53, 297–300. https://doi.org/10.1111/j.1748-5827.2011.01178.x

CHAPTER 2:
SCIENTIFIC AIMS

The overall goal of this research is to contribute to the knowledge base of puppy husbandry practices and selling practices in Belgium, the variation between different breeder types, and the effects thereof on puppy health and behaviour. Breeding practices, such as line-selection and mating strategies, were out of scope of this research. For this purpose, we conducted multiple cross-sectional studies, to answer the following questions:

1. What are the current operating procedures at breeders or breeder merchants in relation to health, hygiene, socialisation, environmental learning, and welfare for breeding dams and puppies in their care? (chapters 3 and 4)

2. Is there a relationship between the origin of the puppy, the type and size of the breeder and the health and behaviour of puppies? (chapters 5 and 6)

3. Can the available scientific information be translated into advices for breeders to ensure the health, socialisation, environmental learning and welfare of their puppies and to comply with the current Belgian legislation? (chapter 7)

CHAPTER 3:
CURRENT SITUATION AT THE DIFFERENT PUPPY BREEDING SYSTEMS WITH REGARD TO SOCIALISATION AND ENVIRONMENTAL LEARNING

This chapter has been adapted from:

On the origin of puppies:

Breeding and selling procedures relevant for canine behavioural development.

Pierre-Alexandre Dendoncker[1,2,3], Tiny De Keuster[1], Claire Diederich[3], DEWULF Jeroen Dewulf[2], Christel P.H. Moons[1]

1 Laboratory for Ethology, Department Nutrition, Genetics and Ethology, Faculty of Veterinary Medicine, Ghent University, Heidestraat 19, 9820 Merelbeke, Belgium

2 Veterinary Epidemiology Unit, Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium

3 Integrated Veterinary Research Unit (IVRU), Department of Veterinary Medicine, Faculty of Sciences, University of Namur, Rue de Bruxelles 61, 5000 Namur, Belgium

Published in *Veterinary Record, 2019,* Volume 184, Issue 710.

http://dx.doi.org/10.1136/vr.104979

1. ABSTRACT

The success of the dog as a companion animal has undeniably led to a shift in dog breeding practices. While effects of inbreeding or large-scale breeding have given rise to numerous studies about potentially related health issues, it remains unclear to what extent behavioural development of dogs is influenced. By investigating the environment of puppies while at the breeder, the authors aimed to make an inventory of current practices regarding management, socialisation and environmental learning and, subsequently, to identify potential differences between breeder types. The cross-sectional study, conducted during 2016, revealed considerable variability in environment among dog breeders. Small-scale breeders, and especially occasional breeders (less than 10 adult dogs on site) provided most enrichment, both social and nonsocial, by, for instance, providing more outdoor access for pregnant dams and puppies or by providing access to visitors more freely. The presentation of environmental stimuli was less controlled in occasional breeders, raising the debate about quantity versus quality of stimuli at a young age. Large-scale breeders declared to screen potential owners less intensely and time to advise them was limited. To the authors' knowledge, this is the first study that compares a large number of environmental factors between the different dog breeding categories.

2. INTRODUCTION

In Western countries, 20-37% of the households own one or more dogs (AVMA, 2012; STATBEL, 2010; Westgarth et al., 2007). In Belgium for example, the total canine population consists of approximately one dog for ten inhabitants, with over 150,000 puppies registered yearly (STATBEL, 2014; FPS Public Health, Unpublished data). Similar figures are seen in the Netherlands (HAS Kennistransfer & Bedrijfsopleidingen, 2015). The main origin of these dogs, aside from private persons (producing approximatively 55% of all puppies), are breeding facilities such as breeding kennels, professional breeders and pet stores (Federal Public Service public health, Unpublished data).

As a result of thousands of years of cohabitation and coevolution, dogs are highly social animals and clearly have been imputed a prominent role in most cultures and societies. In recent centuries, dogs were selected in order to accompany and support human activities and needs (Thalmann et al., 2013). However, consonant with the Post-World War II economic expansion, many dogs have taken up position as a family member (AVMA, 2012; Dotson and Hyatt, 2008; Larson et al., 2012) and were further selected for appearance (i.e., desired physical characteristics such as size, skull shape, coat colour and texture) and behavioural traits. This has resulted in a shift in both breeding purposes and methods, and has unleashed a rush of research on the impact of such a shift on the physical health of several dog breeds (Lindblad-Toh et al., 2005; Sutter and Ostrander, 2004). When it comes to the repercussions of this shift on behavioural development, however, literature is scarce (Gazzano et al., 2008).

The rise in popularity of the dog as a family member has raised the demand for pet dogs, thus creating business opportunities. Currently, breeding facilities range from breeders producing only a few litters per year from a limited breeding stock to breeders procuring hundreds of litters from hundreds of breeding animals. This variety in size is reflected by a corresponding environmental diversity. Typically, during the period at the dog breeder, the puppy's social environment largely depends on the way the facility is managed and consists of the dam and littermates, other animals, caretakers, and visiting humans (Dendoncker et al., 2018). Dogs and humans will play a considerable role in social referencing, thus modelling the response of a

puppy to stimuli and eventually influencing the behaviour at adult age (Merola et al., 2012; Payne et al., 2015). When considering human-dog interactions, not only the bond, but also the experience and knowledge of the caretaker could be of importance (Tami and Gallagher, 2009; Vas et al., 2005). In addition, there is anecdotal evidence that leads one to think that various types of breeders attract a different kind of clientele, which helps explain differences of adult behaviour when retrospectively looking at the origin (Salman et al., 1998; Weng et al., 2006).

Historically, research of canine behavioural development was of great interest, particularly the way in which personality is affected by early environment. Studies provide strong evidence of the effects of insufficient socialisation by stressing the effect on adult behaviour in the absence of sufficient stimuli (Fox, 1971; Fox and Stelzner, 1967; Immelmann and Suomi, 1981; Scott, 1958; Scott and Fuller, 1965), or the importance of the presence of the mother and littermates (Foyer et al., 2013; Wilsson, 1984; Wilsson and Sundgren, 1998) and the risks associated with premature disruption of the mother-offspring bond (Suomi, 1997). In practice, this is often applied by exposing puppies to a large variety of stimuli during the socialisation period, and to breed dogs ideally in a household-like environment.

However, previous studies do not inform about what kind and how much socialisation is optimal (Howell et al., 2015). It is conceivable that there is something like over-stimulation or over-socialisation. While homing of dogs at a later age was shown to be of concern when considering puppies bred in less enriched environments (De Meester et al., 2005; Pfaffenberger and Scott, 1959), it revealed recently to also be associated with a higher prevalence of fear and stranger-directed aggressiveness in puppies bred in a household-like environment (Jokinen et al., 2017; Tiira and Lohi, 2015).

To date, governmental guidelines essentially focus on minimum needs (i.e., housing management: flooring, stocking density, hygiene, vaccination requirements) and medical care (i.e., regulating the contracting veterinarians). However, to support behavioural development appropriately, it is important that these guidelines, for large-scale breeders or other breeder types, reflect the necessities in the field and are not mere transpositions of other settings (Indrebø, 2008).

From the first appearance of commercial breeding facilities, the welfare of breeding dogs has been questioned by the veterinary field and animal welfare associations (Steiger et al., 2008). Certain large-scale breeders were even coined “puppy mill”, characterised by monetary driven breeding of dogs whilst neglecting the physical, psychological or behavioural health of dogs (U.S. District Court for the District of Minnesota, 1984). An increasing number of authors have described that impairments are most likely to occur in puppies from large-scale breeders (Blackwell et al., 2008; Fatjo et al., 2007; Nagasawa et al., 2016; Patronek et al., 1996). Most cross-sectional studies have investigated the odds of compromised behavioural development for dogs born in these facilities through owner reported data (Casey et al., 2014; McMillan et al., 2013; Pirrone et al., 2016).

The relationship between the environment during puppy development and the future behavioural phenotype of the dog does not appear to be straightforward (Strandberg et al., 2005; Tiira and Lohi, 2015). It is not necessarily linked to the satisfaction of the owner or his or her perception of the behaviour (Curb et al., 2013; Serpell, 1996). Events in the owner’s home after the sale cannot be taken out of the equation when testing at later age (Foyer et al., 2014, 2013). Therefore, characterising the variability of key environmental factors in the breeding facility is an important step in elucidating that relationship. At present, there is a lack of comparative research investigating socialisation and environmental enrichment practices at different-sized breeding facilities. A first attempt in this direction was made by De Meester and colleagues, who described the environment in Belgian dog breeding kennels during the puppy socialisation period (De Meester et al., 2005).

Rather than to argue if large-scale breeders provide less environmental stimuli, and whether or not this impairs the ability of dogs bred in these facilities to function adequately in human society, the authors’ focus in the present study is how each breeder category should implement socialisation practices without impacting health and hygiene. Individual breeders may know best practices that have been empirically shown to help puppies develop important life skills in their particular circumstances. However, being able to provide details of these practices could permit researchers and clinicians to assist them to produce pet dogs and dog owners to match expectations and training schemes (Howell and Bennett, 2011).

To the authors' knowledge, clear scientific guidelines that support the behavioural development of dogs in various environments are lacking. Additionally, no data exist that describe how the different breeding facilities need to fulfil the social needs of puppies to maximise the odds for a normal behavioural development. This paper aims to map the current situation regarding management and daily practices, as well as regarding opportunities for socialisation and environmental learning at different types of breeders. Additionally, differences between breeder categories regarding the types of social and nonsocial stimuli provided when raising the puppies and the modalities of a sale were investigated.

3. MATERIALS AND METHODS

Between January 2016 and September 2016, a cross-sectional randomised study was conducted to examine the management conditions among the various breeder types. The target population consisted of all persons breeding or selling dogs in Belgium, which included puppies bred locally as well as those bred abroad and imported. The inclusion criteria for registered and non-registered breeders were that at least one adult dog was present on site and that at least one puppy had been sold during the last two years before the visit. The sampling frame consisted of all licensed breeding facilities and all breeders exempt from being licensed. The former were identified through data made available by the government. The latter were identified via advertisements in magazines, websites, social media, dog breed clubs, and local grocery announcement boards. The sample was stratified according to the seven Belgian legislative categories (*RD 27/04/2007*). A randomised list of breeders within each category was created and the first breeder on each list was contacted by phone to request participation in the study. If a breeder refused, the next breeder on the list was contacted, until the intended sample size (15 per stratum) was reached.

Data were collected by the first author during an on-site orally administered questionnaire (average duration of 55min including a visit of the facility) on all aspects relevant to puppy behavioural development (animals, caretakers, environment, selling process) and observations at the breeding facility. A spreadsheet checklist was developed based on factors described in literature and aspects described in current legislation. More specifically, information was collected on the current number of breeding dams and sires at the time of the enquiry, pregnant

dams, litters (both self-bred and acquired), general management, the structure of the facility and the daily practices, the number and type of people involved in animal care, and exposure of dogs to the social and nonsocial environment. The 544 items (of which 142 were open questions) were pretested and further optimised based on a preliminary visit to three breeding facilities of different categories. The results of the 186 items concerning social and nonsocial environment are further described in this manuscript while data from the remaining 358 questions (concerning hygiene, health and disease management) are described elsewhere (Dendoncker et al., 2018). Selling numbers were reported by the breeder although observations prevailed self-reported data when both were available.

Data were collated in spreadsheet software (Excel 2011, Microsoft Office®). All statistical analyses were done with of SPSS Statistics v24 (IBM SPSS®). Before analysing, skewed continuous data (i.e., dams on site, puppies born yearly) were normalised by means of a log10 transformation. Comparison of the breeder categories with regard to size (number of animals), caretakers and environment was performed by one-way Analysis of Variance (ANOVA) for continuous variables (e.g., the number of animals or the contact time with owners) and Generalised Linear Models were fitted to perform Binary Logistic Regression for binomial variables (e.g., providing outdoor access). Pairwise comparison of the data for each breeder categories with correction for multiple comparisons were performed respectively by Tukey post-hoc analysis for continuous variables and Šidák correction for binomial data. We provided the effect size as Eta Squared (η^2) and the F-Value (F), degrees of freedom (dF) and the statistical significance (corrected P-value) when possible for each continuous variable and we provided the effect size as mean difference (MD), corrected P-Value and Confidence Interval (CI) for each binomial variable. A confidence interval of 95% was retained and significance was retained when a P-value smaller than 0.05 was obtained.

4. RESULTS

During this cross-sectional study, 102 randomly selected dog breeders from all breeder categories were visited. Although the Belgian legislation provides clear categories of breeder types, individual variation between breeders was larger than the intercategorical variation. Therefore, sampled dog breeders were recoded into four categories, according to the number

of dams on site and/or the number of puppies sold yearly. The breeders ranked into the new category showed more similarities (lower intra-category variability and higher inter-category variability) than when ranked into the Belgian categories. The authors decided to retain some terminology (such as occasional) because they will be comprehended by an international audience even without knowledge of the Belgian classification. A detailed description of the alternative breeder categories can be found in box 3.1.

Box 3.1: Alternative breeder categories

Breeder category A is composed of dog breeding facilities limited in size, with less or equal to nine dams on site at the moment of the visit. It is called the category of *occasional dog breeders*. Breeders from category A included all the Belgian legally recognised categories (occasional breeders, hobby breeders, professional breeders and breeder-merchants) with the exception of large breeder-merchants.

Breeder category B: is composed of dog breeding facilities with 10 to 50 dams on site. This category is called the *occupational dog breeders*. Breeders from category B included all legally recognised categories.

Breeder category C is composed of commercial breeding facilities, organised at a scale of 51 or more dams on site. This category is called the *commercial dog breeders.* Breeders from category C included only large professional breeders and large breeder-merchants.

Breeder category D includes merchants with a small-scale breeding facility (equal or more than 50 dams on site), selling equal or more than 350 puppies a year. This category is called the *dog merchants*. Breeders from category D included only breeder-merchants.

Both categories A and B are considered as *small-scale breeders throughout this study* while categories C and D are considered as *large-scale breeders*.

The distribution of the sampled breeders across these four categories and across Belgian categories (discussed in chapter 1) is depicted in table 3.1.

Table 3.1: The number of sampled dog breeders according to the Belgian classification (see chapter 1 for a detailed description) and the alternative classification (A – D).

	Functional categories of dog breeders, based on management practices				Total
	Small-scale		Large-scale		
Legislative categories	Occasional breeder	Occupational breeder	Commercial breeder	Dog merchant	
Occasional Breeder	14	2			16
Small Hobby Breeder	11	4			15
Large Hobby Breeder	7	8			15
Small Professional Breeder	4	11			15
Large Professional Breeder	2	11	2		15
Small Breeder-Merchant	4	5		4	13
Large Breeder-Merchant		4	5	4	13
TOTAL	42	45	7	8	102

Table 3.2 lists the parameters related to the size of a breeding facility per breeder category. Considerable variation in size of the facility was found between the categories. Large-scale breeders were associated with more breeding adults on site ($F_{3,89}$=48.1; P<0.01; η^2=0.6) and selling more puppies ($F_{3,89}$=207.8; P<0.01; η^2=0.9). Commercial breeders had significantly more dams and sires than occupational breeders (MD=0.7; P<0.01; CI=0.4, 0.9), which in turn had more dams and sires than occasional breeders (MD=0.5; P<0.01; CI=0.4, 0.6). The category of dog merchants was represented by merchants having fewer dams and sires than commercial breeders (MD= 0.7; P<0.01; CI=0.4, 1.0), while selling a larger number of dogs than occasional breeders (MD=2.1; P<0.01; CI=1.9, 2.3) and occupational breeders (MD=1.4; P<0.01; CI=1.2, 1.6).

Table 3.2: Number of animals (dams, sires, and puppies) at the different categories of breeders.

	Small-scale		Large-scale	
	Occasional breeder (n=42)	Occupational breeder (n=45)	Commercial breeder (n=7)	Merchant (n=8)
Average number of dams on site (SD)	5.1[a] (2.5)	17.5[b] (8.7)	101.4[c] (51.1)	17.1[b] (17.3)
Dams on site [min; max]	[1; 9]	[10; 34]	[56; 180]	[1; 47]
Average number of sires on site (SD)	2.7[a] (2.0)	6.8[b] (5.4)	16.2[c] (3.8)	4.9[a,b] (4.1)
Sires on site [min; max]	[0; 9]	[0; 23]	[10; 20]	[0; 11]
Average number of puppies born in 2015 (SD)	11.6[a] (12.3)	54.5[b] (54.4)	532.2[c] (307.3)	120.0[b] (72.6)
Puppies born in 2015 [min; max]	[0; 75]	[0 ;300]	[296; 1087]	[30; 200]
Average number of puppies sold in 2015 (SD)	7.2[a] (6.1)	24.6[b] (28.1)	480.0[c] (403.5)	1092.9[c] (638.0)
Puppies sold in 2015 [min; max]	[0; 21]	[0; 100]	[0; 1000]	[500; 2000]

[a-c] Index indicates a significant difference (P<0.05) between the means/percentages

Data about caretakers are listed in table 3.3. Breeder type was associated with the number of caretakers employed ($F_{1,98}$=6.3; P=0.01; η^2=0.06). Large-scale breeders declared to have more caretakers compared to small-scale breeders (MD= 1.9; P=0.01; CI=0.4, 3.4). The ratio of caretakers to the number of dogs on site (adult dogs and puppies) is the highest in occasional breeders (MD=1.6; P<0.01). While large-scale breeders tended to be organised as a company and declared to employ staff more often than small-scale breeders (MD=0.7; P<0.01; CI=0.6, 0.9), a considerable number (46.9%) of breeding facilities consisted of a family operation where, if present, under-aged children (<16 years of age) were involved in the daily care of the dogs. No significant differences arose between the breeder categories regarding the declared involvement of under-aged children (MD=0.2; P=0.06; CI=0.0, 0.5).

Twenty-one breeders (20.6%) declared to have followed a specific education to work with dogs. The most commonly recorded educations related to dogs were grooming (n=10), veterinarian (n=4), veterinary nurse (n=3), and trainer (n=2), while two breeders declared having followed a night school class in dog breeding. Four breeders operated a pig farm besides their dog

breeding activity (A=2, B=1, C=1), five breeders had a stud farm (A=1, B=2, C=1, D=1) and two breeders were previously pig farmers that switched entirely to dog breeding (B=1, C=1).

Table 3.3: Caretakers at the different breeder categories.

	Small-scale		Large-scale	
	Occasional breeder (n=42)	Occupational breeder (n=45)	Commercial breeder (n=7)	Merchant (n=8)
Average (SD) number of caretakers	1.3[a] (1.1)	1.2[a] (0.6)	3.3[b] (1.9)	3.6[b] (2.0)
Breeders where children (<16years) are involved in daily activities (%)	48.8[a]	52.4[a]	50.0[a]	0.0[a]
Breeders with education about dogs (%)	35.7[a]	23.8[a]	14.3[a,b]	0.0[b]

[a-b] Index indicates a significant difference (P<0.05) between the means/percentages

The housing conditions of the dogs at the different breeder categories are depicted in tables 3.4, 3,5, and 3.6. Three physical compartments were observed at all breeders: a location with adult breeding dogs, a maternity ward (dam and litter) and a nursery (where puppies are kept until the sale). Adult breeding animals were mainly kept in pairs or in groups (94.8%), with individual housing being less common among breeders (5.2%, all of which were occasional breeders. 80.6% of all breeders had a separate building unit or room where their adult dogs were housed. Occasional breeders most often (88.1%) declared to provide a household-like environment (i.e., allowing dogs into their own living quarters) for adult breeding dogs compared to occupational breeders (MD=0.3; P<0.01; CI=0.0, 0.5), commercial breeders (MD=0.7; P<0.01; CI=0.4, 1.1) and merchants (MD=0.5; P<0.01; CI=0.0, 1.0). They also declared to provide a household-like environment for their puppies more often compared to commercial (MD=0.6; P=0.04; CI=0.0, 1.0). Occupational breeders declared to provide a household-like environment for adult breeding dogs more often compared to commercial breeders (MD=0.4; P=0.02; CI=0.0, 0.8). Compared to large-scale breeders, small-scale breeders declared to provide more often outdoor access for pregnant dams (MD=0.5; P<0.01; CI=0.3, 0.6) and for puppies (MD=0.6; P<0.01; CI=0.3, 0.8).

The outdoor environment was categorised into solid flooring (e.g., concrete, tiles, pavers or bricks) or in deformable flooring (e.g., grass, sand, or dirt). Large-scale breeders more often declared to limit procedures to daytime for breeding adults (MD=0.5; P<0.01; CI=0.3, 0.6), pregnant dams (MD=0.5; P<0.01; CI=0.4, 0.6) and puppies (MD=0.5; P<0.01; CI=0.4, 0.6).

The social environmental enrichment is defined as contact with other dogs (i.e., dam, littermates, other dogs) and with humans (i.e., caretakers, staff, visitors). On average, pregnant females were declared to be isolated from other adult dogs into a maternity ward after week 7.2 (1.0) of gestation. Sixty-five breeders (63.7%) declared to progressively limit contact between puppies and their mother before weaning. Age of onset for progressive limiting contact in one way or another ranged from birth to seven weeks. Except for one occasional breeder who declared to wean his dogs at five weeks of age, none of the breeders declared to completely wean puppies before eight weeks of age. Overall, 79.8% of all breeders declared to perform contact limitation by relocating the dam, whereas 50.0% relocated the puppies and 41.7% by relocating both. Large-scale breeders showed the highest rate of relocating puppies (MD= 0.4; P<0.01; CI=0.2, 0.7) while no significant differences were found for relocating the dam (MD=0.1; P=0.6; CI=-0.3, 0.2).

Table 3.4: Housing conditions (percentage of breeders) for adult dogs at the different breeder categories

	Small-scale		Large-scale	
	Occasional breeder (n=42)	Occupational breeder (n=45)	Commercial breeder (n=7)	Merchant (n=8)
Breeders providing a household-like environment for adult dogs (%)	88.1[a]	58.1[b]	14.3[c]	37.5[b,c]
Providing access to outdoors for adult dogs (%)	40.6[a,b]	71.4[a]	100.0[a,b]	90.2[b]
Limiting procedures for adult dogs to daytime (%)	35.7[a]	60.0[a,b]	100.0[c]	87.5[b,c]
Granting access for visitors to adult dogs (%)	85.7[a]	54.8[b]	28.6[b]	28.6[b]

[a-c] Index indicates a significant difference (P<0.05) between the percentages

Table 3.5: Housing conditions (percentage of breeders) for pregnant dams at the different breeder categories

	Small-scale		Large-scale	
	Occasional breeder (n=42)	Occupational breeder (n=45)	Commercial breeder (n=7)	Merchant (n=8)
Providing access to outdoors in the maternity ward (%)	87.8[a]	84.4[a]	28.6[b]	37.5[b]
Limiting procedures to daytime in the maternity ward (%)	38.1[a]	57.8[a]	100.0[b]	100.0[b]
Granting access for visitors to maternity ward (%)	42.9[a]	24.4[a]	14.3[a]	12.5[a]

[a-b] Index indicates a significant difference (P<0.05) between the percentages

Table 3.6: Housing conditions (percentage of breeders) for puppies at the different breeder categories

	Small-scale		Large-scale	
	Occasional breeder (n=42)	Occupational breeder (n=45)	Commercial breeder (n=7)	Merchant (n=8)
Providing a household-like environment in the nursery (%)	88.1[a]	65.9[a]	33.3[b]	40.0[a,b]
Providing access to outdoors in the nursery (%)	90.5[a]	84.4[a]	33.3[b]	28.6[b]
Limiting procedures to daytime in the nursery (%)	31.0[a]	60.0[b]	100.0[c]	100.0[c]
Granting access for visitors to nursery (%)	92.7[a]	75.6[a]	28.6[b]	50.0[a,b]

[a-c] Index indicates a significant difference (P<0.05) between the percentages

Table 3.7 lists the types of contact provided by breeders between puppies (before weaning) and adult dogs (different from the dam). Three occasional breeders had no other adult dogs on site, thereby providing limited contact opportunities. Large-scale breeders declared to allow physical contact between puppies and adult dogs significantly less often than small-scale breeders (MD=-0.4; P=0.01; CI=0.1, 0.6), with stimuli limited to olfactory/auditory (only being able to smell/hear other dogs) or visual (being able to smell/hear/see other dogs) without the possibility of direct contact.

Table 3.7: Exposure to environmental stimuli (percentage of breeders) at the different breeder categories

	Small-scale		Large-scale	
	Occasional breeder (n=42)	Occupational breeder (n=45)	Commercial breeder (n=7)	Merchant (n=8)
Breeders who exposed puppies only to olfactory/auditory stimuli (%)	19.4[a]	18.6[a]	42.9[a]	25.0[a]
Breeders who exposed puppies only to visual stimuli (%)	22.2[a]	16.3[a]	28.6[a]	50.0[a]
Breeders who exposed puppies without limitation (%)	58.3[a]	65.1[a]	28.6[b]	25.0[b]

[a-c] Index indicates a significant difference ($P<0.05$) between the percentages

An association was found between occasional breeders and access for visitors to the compartments in which adult dogs were housed and nursery. Occasional breeders granted more access to visitors to the adult dogs compared to occupational breeders (MD= 0.3; $P<0.01$; CI=0.0, 0.6), commercial breeders (MD= 0.6; $P<0.01$; CI=0.1, 1.0) and merchants (MD= 0.6; $P<0.01$; CI=0.1, 1.0). Occupational breeders granted more access to visitors to the adult dogs compared to commercial breeders (MD= 0.6; $P<0.01$; CI=0.2, 1.1). Regarding the maternity ward, occasional breeders declared to allow more contact with visitors than occupational breeders, although this difference was not significant. A showroom (i.e., a compartment of the breeding facility where puppies are housed with free to moderately restricted access to visitors) was observed only at large-scale breeders.

Merchants were predominantly merchants (84.2%) and declared to sell puppies that mainly (80.3%) originated from foreign breeding facilities (i.e., Slovakia and Czech Republic), and to a lesser degree, from other registered Belgian breeding kennels. They also reported selling puppies originating from occasional breeders. Differences between breeder types in reported sale circumstances and provided consumer-advise were investigated. The contact time that breeders reported to spend with potential buyers when selling a puppy ranged from 5 minutes to 240 minutes. Detailed numbers for the contact time can be found in table 3.8. Breeder type was associated with the declared time spend with potential owners ($F_{1,73}=4.1$; $P=0.05$; $\eta^2=0.05$). Large-scale breeders declared to spend less time with new owners than small-scale

breeders (MD=32.3; P=0.04; CI=1.5, 63.2). When comparing the average selling price of breeds in the same *Fédération Cynologique Internationale* breed group (e.g., Terriers or Retrievers, Flushing Dogs and Water Dogs or Sheepdogs and Cattledogs), occupational breeders were observed to charge significantly more than commercial breeders and merchants (P<0.05; η^2=0.2; MD=0.3).

Occasional and occupational breeders declared to screen potential buyers most frequently for composition of the family, spare time, experience with dogs, experience with a particular breed, income, profession, and the general attitude of the buyer towards a dog and its upbringing. Occasional breeders declared to screen potential buyers more often than occupational breeders (MD=0.3; P=0.03; CI=0.0, 0.6), commercial breeders (MD=0.7; P<0.01; CI=0.6, 0.9) and merchants (MD=0.7; P<0.01; CI=0.6, 0.9). Occupational breeders declared to screen potential buyers more often than commercial breeders (MD=0.4; P<0.01; CI=0.2, 0.7) and merchants (MD=0.4; P<0.01; CI=0.2, 0.7). 72.6% of all breeders declared having refused at least once to sell a dog to a particular person. Reasons for refusal included: intended function of the dog, insufficient time to care for the dog, age of the buyer, presence of children in the household, and incorrect expectations towards a particular breed. Forty-two breeders, all of which sold pedigree dogs, reported having a waiting list of candidate-buyers, ranging from three months to up to two years, and five of these breeders required a deposit in order to be enrolled on the list. Five breeders (all large-scale) declared to redirect potential buyers to another breed when asked for a breed not available. Six breeders (four occasional breeders and two occupational breeders) declared to perform behavioural testing to match a puppy with a particular owner.

Table 3.8: Buyer-seller commitment at different breeder categories.

	Small-scale		Large-scale	
	Occasional breeder (n=42)	Occupational breeder (n=45)	Commercial breeder (n=7)	Merchant (n=8)
Average time spent (in min) with new puppy owner (SD)	82.2[a] (47.8)	78.9[a] (52.4)	70.0[b] (17.3)	39.3[b] (21.3)
Percentage (%) of breeders declared to screen potential buyers	74.3[a]	41.9[b]	0.0[c]	0.0[c]

[a-c] Index indicates a significant difference (P<0.05) between the means/percentages

58.8% of all visited breeders declared to provide information to new owners about management, food, behaviour and socialisation, training, prophylaxis, and warranty. The most common types of advice were related to nutrition (94.1%) and health issues (94.1%). The most popular ways among breeders to advise new dog owners were: verbal explanation, either face-to-face or over the telephone (76.5%), on paper (68.6%) or digital handouts (34.3%), and a support website (22.5%). The breeders who reported not to provide any information to owners (n=6) were all occasional breeders.

5. DISCUSSION

The aim of this study was to describe the daily practices relevant for puppy behavioural development, as well as any differences between breeder types concerning management, number of dogs on site, type and number of caretakers, the types of social and nonsocial stimuli provided, and the different aspects that define the selling process, by means of a cross-sectional survey of 102 facilities.

Data of the current study revealed small-scale dog breeders (and especially occasional breeders) more often kept puppies in a household-like environment and were less concerned with maintaining a natural light schedule by limiting the procedures to daytime only. Raising puppies in a household-like environment has anecdotally been accepted in assistance and laboratory dog programs as the best way to ensure dogs are functional in a human society (Howell and Bennett, 2011; Koda, 2001; Loveridge, 1998). Since small-scale breeders also allowed more contact with other dogs and outdoor access in the maternity ward and in the nursery, it can be concluded that they provided a more enriched environment during the socialisation period of puppies than large-scale breeders. Puppies must learn about the environment, i.e., which stimuli can be ignored, and which predict beneficial events or, instead, potentially harmful ones. The effects of lack of such stimulation have been investigated in previous research (Hubrecht, 1993; Scott, 1958; Strandberg et al., 2005). However, the effects of over-stimulation, including stimulation at an intensity exceeding a puppy's threshold for fear, have not been systematically studied (Baskin et al., 2016; Epstein, 2006). A weak stressor must be presented repeatedly in order to achieve habituation while strong stimuli may yield no significant habituation (Grissom and Bhatnagar, 2009; Rankin et al., 2009).

Social interactions are essential for a healthy behavioural development in dogs. In this study, intraspecific, and human familiarisation were the main social interactions investigated. Mother-infant bonding is the formation and maintenance of a social bond between the primary caretaker (mainly the dam) and the offspring and is essentially shaped by the hormone oxytocin (Ainsworth, 1969; Kendrick, 2000; Nagasawa et al., 2012; Young et al., 2001). A shortage in maternal care not only influences behavioural development and stress coping at a later age, as shown in rodent models (Liu et al., 2000, 1997), but also impairs spatial learning and memory (Liu et al., 2000; Meaney, 2001). As a general accepted rule, weaning should not be completed until seven weeks of age (Wilsson, 1984).

Intraspecific socialisation of puppies occurs through contact with the dam, the littermates, and other dogs (Slabbert and Rasa, 1997). In the presented data, no associations were found between the breeder category and the contact time or the frequency of contact between the dam and her offspring, or how the breeder realises the separation, except the frequency of relocating weaned puppies. It seems likely that awareness for the minimal weaning age has grown since the publication of De Meester and colleagues in which 22 out of 62 breeders reported weaning the puppies before seven weeks of age (De Meester et al., 2005). Large-scale breeders provided fewer intraspecific interactions for puppies (i.e., contact with adult dogs other than the dam) than small-scale breeders. Although enrichment guidelines focus on the provision of visual contact between dogs (Wells, 2004), it is conceivable that limiting physical contact (i.e., to limit introduction and spread of pathogens) may influence the socialisation process (Previde et al., 2009; Tuber et al., 1996).

Interspecific socialisation with humans occurs mainly through contact with the principal caretaker and other humans the puppy comes in contact with. Differences regarding numbers of caretakers were recorded, and although large-scale breeders more often employed caretakers, it appeared that the ratio of animals (dams and puppies) to caretakers was the lowest for occasional breeders, which could result in more socialisation opportunities as well as more social bonding between the caretaker and the dog. However, interpretation of this data is difficult since this does not take into account the number of man-hours a day spent per dog nor the quality of interaction with the dog.

Dog breeders varied in knowledge and background across all breeder categories. While it is often said that some large-scale breeders are previous farmers that switched to dog breeding to maximise profits (Tushaus, 2009), we found retired and active farmers in all types of breeders.

Interestingly, data in this study showed differences in whether and how often visitors are allowed, with occasional breeders appearing to be less strict in prohibiting contact from visitors. Biosecurity measures and the scale of a facility taken into account, restricting access for strangers is common in large-scale breeders, and more incidental for small-scale breeders. It can be hypothesised that some breeders prefer not to disclose the housing conditions of their dogs and therefore restrict visitors to the showroom area. Another hypothesis for access restriction is to minimise stress/fear in the dam. The question of whether or not a pregnant dam will respond fearfully and/or stressed in the presence of a stranger, depends on many factors. It is related to individual differences (Hennessy et al., 2001), is breed dependent (Svartberg, 2006), and depends on the type of communication and the perception by the animal (Vas et al., 2005). This can be partially explained by the social referencing in dog-human interactions: the caretakers' reaction could influence the response of a dam (Merola et al., 2012). Recent research has shown that bonding with humans can result in a secure base effect (i.e., a buffer against stress), and is generally directed at the owner but sporadically at the primary caretaker or even unfamiliar humans (Horn et al., 2013; Nagasawa et al., 2009). Evidence in several species revealed that the exposure of the dam to unpredictable prenatal stress impacts the neuroendocrine development and may selectively degrade social behaviour of the offspring (Glover and O'Connor, 2002; Huizink et al., 2003; Lee et al., 2007; Weinstock, 1997). In order to enable the dogs to cope with stress, they should be provided with meaningful control over their environment, especially around parturition (Rooney et al., 2009). Since some breeding dogs may find human encounters stressful (McMillan et al., 2011), a possible solution, in analogy to zoo animals, could be altering the conditions of housing (e.g., providing hide-outs) to give the dam (and puppies at a later age) more control over their encounters with strangers (Hosey, 2005). While social withdrawal is to be expected shortly before parturition, some dams are segregated for weeks around parturition date (Dendoncker et al., 2018). It can be hypothesised that providing positive intraspecific social interactions can be beneficial for the mental health of

breeding dams. Further research on social referencing at different breeder types is needed to determine if interaction with strangers in the context of a maternity ward is an added value for the dam or contrarily, has a negative effect.

Data presented in this paper illustrate differences between the buyers at the different breeder types. Buyers from occupational breeders (i.e., Kennel Club) are willing to wait for a puppy, have their life and family situation screened, and are prepared to buy the puppy for a considerably higher price. It can be hypothesised that these buyers are better prepared and judicious when it comes to buying and raising a puppy, thus reducing the risk of future behavioural problems and/or shelter relinquishment. In contrast, merchants are perceived as low-threshold, less expensive, and, most-often, the desired puppy is 'in stock'. But even if this is not the case, people visiting large-scale breeders can be guided to buy and indeed acquire another breed they originally intended to. This finding confirms that the origin of the dog is often subjected to confounders, some of which are related to the owner (Pirrone et al., 2016). All owners are not equally prepared to take a puppy into their home (Endersby and Health, 2013). Therefore, the different types of owners should be taken into account when comparing different types of dog breeders through owner-reported behaviour. How the owners perceive the behaviour of their dog is not only related to previous experiences, but is also dictated by investment (Collisson, 2015), bonding (Meyer and Forkman, 2014), expectations about the care and costs of having a dog, and further sociodemographic data of the owner (O'Connor et al., 2016; Patronek et al., 1996).

Verbal communication is the most popular way of sharing information when selling a dog in all categories, but the ability to process the information will vary based on the experience and socioeconomic status of the potential buyers (Childers et al., 1985). Paradoxically, some small-scale breeders declared to give no advice to new owners, namely occasional breeders. It is important to notice that, for registered breeders, there is an obligation to provide a minimum of advice (*RD 27/04/2007*). Occasional breeders are thus not obliged to inform potential owners. However, it can be hypothesised that some household breeders lack the knowledge to give proper advice to potential buyers. Also, it can be hypothesised that some occasional breeders operate for profit, neglecting their role in consumer advice, and because of their small-scale,

they remain unmonitored and unregulated. This type of breeder has been referred to in literature as backyard breeders and could be an increasing problem regarding animal welfare.

Additionally, large-scale breeders operate in a more restricted timeframe when selling dogs, whereby, according to our data, only the most relevant information was passed on, forsaking seemingly 'less important' information. It is to be expected, therefore, that not every buyer is sufficiently informed or experienced, increasing the risk of future behavioural problems or shelter relinquishment.

There are a number of limitations to this study. First, although a randomised selection was applied, collaboration was achieved only by willingness to participate. As other epidemiological studies have shown, self-selection may introduce systematic errors in prevalence estimates as well as in association measures (Nilsen et al., 2009). As a result, questionable breeding practices might be underrepresented, and some breeders could be especially opposed to allowing strangers in to observe their facilities. However, given that dog breeding is currently an emotional and debated subject, willingness to participate was deemed to be a more reliable way of gaining certain information that would not be obtainable through a mandatory assessment by governmental audit. The main reasons not to participate were described elsewhere (Dendoncker et al., 2018). Second, since there is no official quantification of non-accredited breeders and no databases are available, the total population is unknown. To limit the risk of selection bias, a multi-stage sampling approach was applied.

6. CONCLUSION

This study provides a detailed description of different canine breeding facilities and permitted a comparison between the distinct groups of breeders, based on demographics, with regard to management, opportunities for socialisation and environmental learning, and in terms of the sale of a puppy. Large-scale breeders appeared to be most rigorous regarding disease prevention and management, stressing the principles of limitation and control of disease in the breeding facility. As a result however, this breeder category provided a less enriched environment, both social and nonsocial compared to small-scale breeders. In occasional breeders, the question arises if more control of the environmental stimuli could be deemed profitable in order to avoid over-stimulation of puppies and to limit non-controlled exposure of a nursing dam. Compared to occupational breeders, large-sale breeders appeared to target a different clientele, by demanding a lower commitment from puppy buyers, both time wise and financially. Differences between the breeder types, as presented in this paper, are an important factor to take into account when drawing up practical guidelines on socialisation, environmental enrichment, and welfare assessment. This study provides a foundation to identify and validate possible causative factors in behavioural development and establishes the basis for subsequent investigation of behavioural differences between puppies originating from different breeders.

7. REFERENCES

Ainsworth, M.D.S., 1969. Object Relations, Dependency, and Attachment: A Theoretical Review of the Infant-Mother Relationship. Child Dev. 40, 969. https://doi.org/10.2307/1127008

AVMA, 2012. U.S. pet ownership and demographics sourcebook. American Veterinary Medical Association, Schaumburg, IL.

Baskin, S., Zamansky, A., Kononova, V., 2016. Exploring human perceptions of dog-tablet playful interactions, in: Proceedings of the Third International Conference on Animal-Computer Interaction.

Blackwell, E.J., Twells, C., Seawright, A., Casey, R.A., 2008. The relationship between training methods and the occurrence of behavior problems, as reported by owners, in a population of domestic dogs. J. Vet. Behav. Clin. Appl. Res. 3, 207–217. https://doi.org/10.1016/j.jveb.2007.10.008

Casey, R.A., Loftus, B., Bolster, C., Richards, G.J., Blackwell, E.J., 2014. Human directed aggression in domestic dogs (Canis familiaris): Occurrence in different contexts and risk factors. Appl. Anim. Behav. Sci. 152, 52–63. https://doi.org/10.1016/j.applanim.2013.12.003

Childers, T.L., Houston, M.J., Heckler, S.E., E., H.S., 1985. Measurement of Individual Differences in Visual Versus Verbal Information Processing. J. Consum. Res. 12, 125–134. https://doi.org/10.2307/254345

Collisson, B., 2015. Farewell to Fido : Pet Owners ' Commitment and Relinquishment of Companion Animals. Am. J. Appl. Psychol. 3, 27–33. https://doi.org/10.12691/ajap-3-2-1

Curb, L.A., Abramson, C.I., Grice, J.W., Kennison, S.M., 2013. The Relationship between Personality Match and Pet Satisfaction among Dog Owners. Anthrozoos 26, 395–404. https://doi.org/10.2752/175303713X13697429463673

De Meester, R., Moons, C., van Bree, H., Coopman, F., 2005. Critical evaluation of the environment in Belgian dog breeding kennels during the puppies' socialization period. Vlaams Diergeneeskd. Tijdschr. 74, 364–374.

Dendoncker, P.-A., Moons, C., Sarrazin, S., Diederich, C., Thiry, E., de Keuster, T., Dewulf, J., 2018. Biosecurity and management practices in different dog breeding systems have considerable margin for improvements. Vet. Rec. 183. https://doi.org/10.1136/vr.104996

Dotson, M.J., Hyatt, E.M., 2008. Understanding dog–human companionship. J. Bus. Res. 61, 457–466. https://doi.org/10.1016/j.jbusres.2007.07.019

Endersby, S., Health, A., 2013. Buying on impulse? Survey highlights puppy owners' lack of research. Vet. Rec. 172, 408.

Epstein, L.A., 2006. There Are Not Bad Dogs, Only Bad Owners: Replacing Strict Liability with a Negligence Standard in Dog Bite Cases. Anim. Law 13, 129–146.

Fatjo, J., Amat, M., Mariotti, V.M., de la Torre, J.L.R., Manteca, X., 2007. Analysis of 1040 cases of canine aggression in a referral practice in Spain. J. Vet. Behav. Clin. Appl. Res. 2, 158–165. https://doi.org/10.1016/j.jveb.2007.07.008

Fox, M.W., 1971. Integrative development of brain and behavior in the dog. University of Chicago Press, Chicago.

Fox, M.W., Stelzner, D., 1967. The effects of early experience on the development of inter and intraspecies social relationships in the dog. Anim. Behav. 15, 377–386. https://doi.org/10.1016/0003-3472(67)90024-3

Foyer, P., Bjällerhag, N., Wilsson, E., Jensen, P., 2014. Behaviour and experiences of dogs during the first year of life predict the outcome in a later temperament test. Appl. Anim. Behav. Sci. 155, 93–100. https://doi.org/10.1016/j.applanim.2014.03.006

Foyer, P., Wilsson, E., Wright, D., Jensen, P., 2013. Early experiences modulate stress coping in a population of German shepherd dogs. Appl. Anim. Behav. Sci. 146, 79–87. https://doi.org/10.1016/J.APPLANIM.2013.03.013

Gazzano, A., Mariti, C., Notari, L., Sighieri, C., McBride, E.A., 2008. Effects of early gentling and early environment on emotional development of puppies. Appl. Anim. Behav. Sci. 110, 294–304. https://doi.org/10.1016/j.applanim.2007.05.007

Glover, V., O'Connor, T.G., 2002. Effects of antenatal stress and anxiety: Implications for development and psychiatry. Br. J. Psychiatry 180, 389–91. https://doi.org/10.1192/BJP.180.5.389

Grissom, N., Bhatnagar, S., 2009. Habituation to repeated stress: get used to it. Neurobiol. Learn. Mem. 92, 215–224. https://doi.org/10.3174/ajnr.A1256.Functional

HAS Kennistransfer & Bedrijfsopleidingen, 2015. Feiten & Cijfers Gezelschapsdierensector 2015, HAS Hogeschool & Utrecht University faculty of Veterinary medicine. Den Bosch.

Hennessy, M., Voith, V., Mazzei, S., … J.B.-A.A., 2001, U., 2001. Behavior and cortisol levels of dogs in a public animal shelter, and an exploration of the ability of these measures to predict problem behavior after adoption. Appl. Anim. Behav. Sci. 73, 217–233.

Horn, L., Huber, L., Range, F., 2013. The Importance of the Secure Base Effect for Domestic Dogs - Evidence from a Manipulative Problem-Solving Task. PLoS One 8, e65296. https://doi.org/10.1371/journal.pone.0065296

Hosey, G.R., 2005. How does the zoo environment affect the behaviour of captive primates? Appl. Anim. Behav. Sci. 90, 107–129. https://doi.org/10.1016/J.APPLANIM.2004.08.015

Howell, T., King, T., Bennett, P., 2015. Puppy parties and beyond: the role of early age socialization practices on adult dog behavior. Vet. Med. Res. Reports 6, 143. https://doi.org/10.2147/VMRR.S62081

Howell, T.J., Bennett, P.C., 2011. Puppy power! Using social cognition research tasks to improve socialization practices for domestic dogs (Canis familiaris). J. Vet. Behav. Clin. Appl. Res. 6, 195–204. https://doi.org/10.1016/j.jveb.2011.01.004

Hubrecht, R.C., 1993. A comparison of social and environmental enrichment methods for laboratory housed dogs. Appl. Anim. Behav. Sci. 37, 345–361. https://doi.org/10.1016/0168-1591(93)90123-7

Huizink, A.C., Robles de Medina, P.G., Mulder, E.J.H., Visser, G.H.A., Buitelaar, J.K., 2003. Stress during pregnancy is associated with developmental outcome in infancy. J. Child Psychol. Psychiatry 44, 810–818. https://doi.org/10.1111/1469-7610.00166

Immelmann, K., Suomi, S.J., 1981. Sensitive phases in development, Behavioral Development. Cambridge University Press.

Indrebø, A., 2008. Animal welfare in modern dog breeding. Acta Vet. Scand. 50, S6. https://doi.org/10.1186/1751-0147-50-S1-S6

Jokinen, O., Appleby, D., Sandbacka-Saxén, S., Appleby, T., Valros, A., 2017. Homing age influences the prevalence of aggressive and avoidance-related behaviour in adult dogs. Appl. Anim. Behav. Sci. 195, 87–92. https://doi.org/10.1016/J.APPLANIM.2017.06.003

Kendrick, K.M., 2000. Oxytocin, motherhood and bonding. Exp. Physiol. 85, 111s–124s. https://doi.org/10.1111/j.1469-445X.2000.tb00014.x

Koda, N., 2001. Development of play behavior between potential guide dogs for the blind and human raisers. Behav. Processes 53, 41–46. https://doi.org/10.1016/S0376-6357(00)00146-7

Larson, G., Karlsson, E.K., Perri, A., Webster, M.T., Ho, S.Y.W., Peters, J., Stahl, P.W., Piper, P.J., Lingaas, F., Fredholm, M., Comstock, K.E., Modiano, J.F., Schelling, C., Agoulnik, A.I., Leegwater, P.A., Dobney, K., Vigne, J.-D., Vila, C., Andersson, L., Lindblad-Toh, K., 2012. Rethinking dog domestication by integrating genetics, archeology, and biogeography. Proc. Natl. Acad. Sci. 109, 8878–8883. https://doi.org/10.1073/pnas.1203005109

Lee, P.R., Brady, D.L., Shapiro, R.A., Dorsa, D.M., Koenig, J.I., 2007. Prenatal stress generates deficits in rat social behavior: Reversal by oxytocin. Brain Res. 1156, 152–167. https://doi.org/10.1016/j.brainres.2007.04.042

Lindblad-Toh, K., Wade, C.M., Mikkelsen, T.S., Karlsson, E.K., Jaffe, D.B., Kamal, M., Clamp, M., Chang, J.L., Kulbokas, E.J., Zody, M.C., 2005. Genome sequence, comparative

analysis and haplotype structure of the domestic dog. Nature 438, 803–819. https://doi.org/10.1038/nature04338

Liu, D., Diorio, J., Day, J.C., Francis, D.D., Meaney, M.J., 2000. Maternal care, hippocampal synaptogenesis and cognitive development inrats. Nat. Neurosci. 3, 799–806. https://doi.org/10.1038/77702

Liu, D., Diorio, J., Tannenbaum, B., Caldji, C., Francis, D., Freedman, A., Sharma, S., Pearson, D., Plotsky, P.M., Meaney, M.J., 1997. Maternal Care, Hippocampal Glucocorticoid Receptors, and Hypothalamic-Pituitary-Adrenal Responses to Stress. Science (80-.). 277, 1659–1662.

Loveridge, G.., 1998. Environmentally enriched dog housing. Appl. Anim. Behav. Sci. 59, 101–113. https://doi.org/10.1016/S0168-1591(98)00125-7

McMillan, F.D., Duffy, D.L., Serpell, J.A., 2011. Mental health of dogs formerly used as "breeding stock" in commercial breeding establishments. Appl. Anim. Behav. Sci. https://doi.org/10.1016/j.applanim.2011.09.006

McMillan, F.D., Serpell, J.A., Duffy, D.L., Masaoud, E., Dohoo, I.R., 2013. Differences in behavioral characteristics between dogs obtained as puppies from pet stores and those obtained from noncommercial breeders. J. Am. Vet. Med. Assoc. 242, 1359–1363. https://doi.org/10.2460/javma.242.10.1359

Meaney, M.J., 2001. Maternal Care, Gene Expression, and the Transmission of Individual Differences in Stress Reactivity Across Generations. Annu. Rev. Neurosci. 24, 1161–1192. https://doi.org/10.1146/annurev.neuro.24.1.1161

Merola, I., Prato-Previde, E., Marshall-Pescini, S., 2012. Dogs' Social Referencing towards Owners and Strangers. PLoS One 7, e47653. https://doi.org/10.1371/journal.pone.0047653

Meyer, I., Forkman, B., 2014. Dog and owner characteristics affecting the dog–owner relationship. J. Vet. Behav. Clin. Appl. Res. 9, 143–150. https://doi.org/10.1016/j.jveb.2014.03.002

Nagasawa, M., Kanbayashi, S., Mogi, K., Serpell, J.A., Kikusui, T., 2016. Comparison of behavioral characteristics of dogs in the United States and Japan. J. Vet. Med. Sci. 78, 231–8. https://doi.org/10.1292/jvms.15-0253

Nagasawa, M., Kikusui, T., Onaka, T., Ohta, M., 2009. Dog's gaze at its owner increases owner's urinary oxytocin during social interaction. Horm. Behav. 55, 434–441. https://doi.org/https://doi.org/10.1016/j.yhbeh.2008.12.002

Nagasawa, M., Okabe, S., Mogi, K., Kikusui, T., 2012. Oxytocin and mutual communication in mother-infant bonding. Front. Hum. Neurosci. 6, 1–10. https://doi.org/10.3389/fnhum.2012.00031

Nilsen, R.M., Vollset, S.E., Gjessing, H.K., Skjaerven, R., Melve, K.K., Schreuder, P., Alsaker, E.R., Haug, K., Daltveit, A.K., Magnus, P., 2009. Self-selection and bias in a large prospective pregnancy cohort in Norway. Paediatr. Perinat. Epidemiol. 23, 597–608. https://doi.org/10.1111/j.1365-3016.2009.01062.x

O'Connor, R., Coe, J.B., Niel, L., Jones-Bitton, A., 2016. Effect of Adopters' Lifestyles and Animal-Care Knowledge on Their Expectations Prior to Companion-Animal Guardianship. J. Appl. Anim. Welf. Sci. 19, 157–170. https://doi.org/10.1080/10888705.2015.1125295

Patronek, G.J., Glickman, L.T., Beck, A.M., McCabe, G.P., Ecker, C., 1996. Risk factors for relinquishment of dogs to an animal shelter. J. Am. Vet. Med. Assoc. 209, 572–81.

Payne, E., Bennett, P., McGreevy, P., 2015. Current perspectives on attachment and bonding in the dog–human dyad. Psychol. Res. Behav. Manag. 71. https://doi.org/10.2147/PRBM.S74972

Pfaffenberger, C.J., Scott, J.P., 1959. The Relationship between Delayed Socialization and Trainability in Guide Dogs. J. Genet. Psychol. 95, 145–155. https://doi.org/10.1080/00221325.1959.10534251

Pirrone, F., Pierantoni, L., Pastorino, G.Q., Albertini, M., 2016. Owner-reported aggressive behavior towards familiar people may be a more prominent occurrence in pet shop-traded dogs. J. Vet. Behav. Clin. Appl. Res. 11, 13–17. https://doi.org/10.1016/j.jveb.2015.11.007

Previde, E.P., Ghirardelli, G., Marshall-Pescini, S., Valsecchi, P., 2009. Intraspecific attachment in domestic puppies (Canis familiaris). J. Vet. Behav. Clin. Appl. Res. 4, 89–90. https://doi.org/10.1016/J.JVEB.2008.10.002

Rankin, C.H., Abrams, T., Barry, R.J., Bhatnagar, S., Clayton, D., Colombo, J., Coppola, G., Geyer, M.A., Glanzman, D.L., Marsland, S., Mcsweeney, F., Wilson, D.A., Wu, C., Thompson, R.F., 2009. Habituation Revisited: An Updated and Revised Description of the Behavioral Characteristics of Habituation Catharine. Neurobiol. Learn. Mem. 92, 135–138. https://doi.org/10.1016/j.nlm.2008.09.012.Habituation

Rooney, N., Gaines, S., Hiby, E., 2009. A practitioner's guide to working dog welfare. J. Vet. Behav. Clin. Appl. Res. 4, 127–134. https://doi.org/10.1016/j.jveb.2008.10.037

Salman, M.D., New, Jr., J.G., Scarlett, J.M., Kass, P.H., Ruch-Gallie, R., Hetts, S., 1998. Human and Animal Factors Related to Relinquishment of Dogs and Cats in 12 Selected Animal Shelters in the United States. J. Appl. Anim. Welf. Sci. 1, 207–226. https://doi.org/10.1207/s15327604jaws0103_2

Scott, J., 1958. Critical periods in the development of social behavior in puppies. Psychosom. Med. 20, 42–54.

Scott, J.P., Fuller, J.L., 1965. Genetics and the Social Behavior of the Dog. University of Chicago Press, Chicago.

Serpell, J.A., 1996. Evidence for an association between pet behavior and owner attachment levels. Appl. Anim. Behav. Sci. 47, 49–60. https://doi.org/10.1016/0168-1591(95)01010-6

Slabbert, J.M., Rasa, O.A.E., 1997. Observational learning of an acquired maternal behaviour pattern by working dog pups: an alternative training method? Appl. Anim. Behav. Sci. 53, 309–316. https://doi.org/10.1016/S0168-1591(96)01163-X

STATBEL, 2010. De honden en katten van de Belgen [WWW Document]. URL http://statbel.fgov.be/nl/binaries/De honden en katten van de Belgen_2008_nl_tcm325-103755.pdf

Steiger, A., Stucki, F., Peyer, N., Keller, P., 2008. [Assessment of animal welfare aspects in extreme breeds of pet animals]. Schweiz. Arch. Tierheilkd. 150, 217–25. https://doi.org/10.1024/0036-7281.150.5.217

Strandberg, E., Jacobsson, J., Saetre, P., 2005. Direct genetic, maternal and litter effects on behaviour in German shepherd dogs in Sweden. Livest. Prod. Sci. 93, 33–42. https://doi.org/10.1016/j.livprodsci.2004.11.004

Suomi, S.J., 1997. Early determinants of behaviour: evidence from primate studies. Br. Med. Bull. 53, 170–184. https://doi.org/10.1093/oxfordjournals.bmb.a011598

Sutter, N.B., Ostrander, E.A., 2004. Dog star rising: the canine genetic system. Nat. Rev. Genet. 5, 900–910.

Svartberg, K., 2006. Breed-typical behaviour in dogs—Historical remnants or recent constructs? Appl. Anim. Behav. Sci. 96, 293–313. https://doi.org/https://doi.org/10.1016/j.applanim.2005.06.014

Tami, G., Gallagher, A., 2009. Description of the behaviour of domestic dog (Canis familiaris) by experienced and inexperienced people. Appl. Anim. Behav. Sci. 120, 159–169. https://doi.org/10.1016/j.applanim.2009.06.009

Thalmann, O., Shapiro, B., Cui, P., Schuenemann, V.J., Sawyer, S.K., Greenfield, D.L., Germonpré, M.B., Sablin, M. V., López-Giráldez, F., Domingo-Roura, X., Napierala, H., Uerpmann, H.P., Loponte, D.M., Acosta, A.A., Giemsch, L., Schmitz, R.W., Worthington, B., Buikstra, J.E., Druzhkova, A., Graphodatsky, A.S., Ovodov, N.D., Wahlberg, N., Freedman, A.H., Schweizer, R.M., Koepfli, K.P., Leonard, J.A., Meyer, M., Krause, J., Pääbo, S., Green, R.E., Wayne, R.K., 2013. Complete mitochondrial genomes of ancient canids suggest a

European origin of domestic dogs. Science (80-.). 342, 871–874. https://doi.org/10.1126/science.1243650

Tiira, K., Lohi, H., 2015. Early Life Experiences and Exercise Associate with Canine Anxieties. PLoS One 10, e0141907. https://doi.org/10.1371/journal.pone.0141907

Tuber, D.S., Hennessy, M.B., Sanders, S., Miller, J.A., 1996. Behavioral and glucocorticoid reponses of adult domestic dogs (Canis familiaris) to companionship and social separation. J. Comp. Psychol. 110, 103–108. https://doi.org/10.1037/0735-7036.110.1.103

Tushaus, K.C., 2009. Don't Buy the Doggy in the Window: Ending the Cycle that Perpetuates Commercial Breeding with Regulation of the Retail Pet Industry. Drake J. Agric. Law 14, 501.

U.S. District Court for the District of Minnesota, 1984. Avenson v. Zegart, 577 F. Supp. 958 (D. Minn. 1984) :: Justia [WWW Document]. URL https://law.justia.com/cases/federal/district-courts/FSupp/577/958/1497077/ (accessed 1.16.18).

Vas, J., Topál, J., Gácsi, M., Miklósi, Á., Csányi, V., Miklósi, A., Csányi, V., 2005. A friend or an enemy? Dogs' reaction to an unfamiliar person showing behavioural cues of threat and friendliness at different times. Appl. Anim. Behav. 94, 99–115. https://doi.org/10.1016/j.applanim.2005.02.001

Weinstock, M., 1997. Does Prenatal Stress Impair Coping and Regulation of Hypothalamic-Pituitary-Adrenal Axis? Neurosci. Biobehav. Rev. 21, 1–10. https://doi.org/https://doi.org/10.1016/S0149-7634(96)00014-0

Wells, D.L., 2004. A review of environmental enrichment for kennelled dogs, Canis familiaris. Appl. Anim. Behav. Sci. 85, 307–317. https://doi.org/10.1016/j.applanim.2003.11.005

Weng, H.Y., Kass, P.H., Hart, L.A., Chomel, B.B., 2006. Risk factors for unsuccessful dog ownership: An epidemiologic study in Taiwan. Prev. Vet. Med. 77, 82–95. https://doi.org/10.1016/j.prevetmed.2006.06.004

Westgarth, C., Pinchbeck, G.L., Bradshaw, J.W., Dawson, S., Gaskell, R.M., Christley, R.M., 2007. Factors associated with dog ownership and contact with dogs in a UK community. BMC Vet. Res. 3, 5. https://doi.org/10.1186/1746-6148-3-5

Wilsson, E., 1984. The social interaction between mother and offspring during weaning in German shepherd dogs: individual differences between mothers 1. Wilsson E. 1984; The social interaction between mother and offspring during weaning in German shepherd dogs: individual d. Appl. Anim. Behav. Sci. 13, 101–112. https://doi.org/https://doi.org/10.1016/0168-1591(84)90056-X

Wilsson, E., Sundgren, P.-E., 1998. Effects of weight, litter size and parity of mother on the behaviour of the puppy and the adult dog. Appl. Anim. Behav. Sci. 56, 245–254. https://doi.org/https://doi.org/10.1016/S0168-1591(97)00094-4

Young, L.J., Lim, M.M., Gingrich, B., Insel, T.R., 2001. Cellular Mechanisms of Social Attachment. Horm. Behav. 40, 133–138. https://doi.org/https://doi.org/10.1006/hbeh.2001.1691

CHAPTER 4:
CURRENT SITUATION AT THE DIFFERENT PUPPY BREEDING SYSTEMS WITH REGARD TO BIOSECURITY AND HEALTH MANAGEMENT

This chapter has been adapted from:

Biosecurity and management practices in different dog breeding systems have considerable margin for improvements.

Pierre-Alexandre Dendoncker[1,2,3], Christel P.H. Moons[1], Steven Sarrazin[2], Claire Diederich[3], Etienne Thiry[4], Tiny De Keuster[1], Jeroen Dewulf[2]

1 Laboratory for Ethology, Department of Nutrition, Genetics and Ethology, Faculty of Veterinary Medicine, Ghent University, Heidestraat 19, 9820 Merelbeke

2 Veterinary Epidemiology Unit, Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke

3 Integrated Veterinary Research Unit (IVRU), Department of Veterinary Medicine, Faculty of Sciences, University of Namur, Rue de Bruxelles 61, 5000 Namur

4 Virology, Department of Infectious and Parasitic Diseases, FARAH Centre, Faculty of Veterinary Medicine, University of Liège, Avenue de Cureghem, 10, 4000 Liège, Belgium

Published in Veterinary Record, 2018, Volume 183, Issue 381.

http://dx.doi.org/10.1136/vr.104996

1. ABSTRACT

To investigate the current management and biosecurity practices and identify possible differences between different types of breeders, a cross-sectional study was carried out in 102 Belgian dog breeding facilities ranging from small (less than 10 dams on site) to large-scale (more than 50 dams on site or at least 350 puppies sold yearly) breeders.

Veterinary prophylactic protocols (i.e., vaccination, endoparasite control, ectoparasitic treatments) were mostly highly implemented (91.5%, 92.6%, 42.7% respectively) across all breeder categories. 13.8% of all visited breeders reported to administer antimicrobials to each female post-partum and 10.3% reported to treat all puppies, or at least of one breed, systematically with antimicrobials.

Large-scale breeders reported to employ staff more frequently (MD=0.7; P<0.01; CI=0.6, 0.9), and appeared to be more familiar with the principles of biosecurity. Compared to small-scale breeders, they reported to apply disinfection more often at the adult dogs (MD=0.6; P<0.01; CI=0.4, 0.8) and at the maternity ward (MD=0.5; P<0.01; CI=0.2, 0.7). They also reported to apply hygienic measures more often at the maternity ward (MD=0.4; P<0.01; CI=0.1, 0.7) and the nursery (MD=0.3; P=0.04; CI=0.0, 0.5) and had a tendency to apply hygienic measures more often at the adult dogs (MD=0.2; P=0.08; CI=0.0, 0.5) and to quarantine newly acquired dogs (MD=0.7; P<0.01; CI=0.5, 0.9) more often compared to small-scale breeders. Nonetheless, a moderate knowledge of and use of disinfection was recorded, as was the presence of pet dogs, breaking the compartmentalisation. Results of this study indicate that there is substantial room for improvement in hygiene and disease management across all categories of breeders.

The characterisation of different types of dog breeders with respect to biosecurity and management practices is a first step towards improvement of dog husbandry and biosecurity measures. Tailored guidelines should permit breeders to further improve the health of breeding animals and puppies while reducing the risk of infectious disease outbreaks and associated expenses.

2. INTRODUCTION

In the past decennia, operations of large-scale canine breeders (i.e., selling more than 20 litters a year) and merchants have been linked to higher incidences of illness and disease outbreaks in dogs compared to those of small-scale breeders (Bandai et al., 1999; Hird et al., 1992; Scarlett et al., 1994; Schumaker et al., 2012; Stavisky et al., 2012; Stehr-Green and Murray, 1987), possibly obstructing the welfare of puppies and breeding dogs. Improvement in prophylactic treatments and immunisation might be expected due to the efforts of expert groups publishing and distributing guidelines in the last decade (Day et al., 2016; ESCCAP, 2010a; Looney et al., 2008; Welborn et al., 2011).

However, despite available guidelines, there is evidence that applied prophylactic protocols for puppies are insufficient for good dog breeding facility management. Several authors have suggested that immunoprophylaxis against common canine viral pathogens can fail because of incorrect application (Day et al., 2007), insufficient immunisation (Ellis, 2010) or interference with maternal immunity (Decaro and Buonavoglia, 2012; Thiry and Horzinek, 2007). Endoparasite control is widely applied on a regular basis, however routinely used formulations are not effective against *Giardia* spp*. and Cystoisospora* spp., and high prevalences of these endoparasites are reported in breeding facilities (Dupont et al., 2013). Although there is no evidence yet, it is possible that the current endoparasite control strategies might hasten the appearance of benzimidazole resistance at dog breeding facilities, as has occurred in livestock (Bugg et al., 1999).

The periparturient period is undoubtedly the period with the highest risk of disease transmission, and antimicrobial treatments (e.g., amoxicillin-clavulanic acid or cephalosporins) are readily available and applied during this period (Milani et al., 2012; Münnich, 2008). Excessive and incorrect implementation of antimicrobials may, contrarily to their purpose, give rise to unwanted or even dangerous circumstances such as the development of drug resistance. Antimicrobial use in small animals has been identified as one of the risk factors for colonisation or infection with resistant pathogens (Bramble et al., 2011; De Graef et al., 2004; Pomba et al., 2016; Rota et al., 2011).

In analogy with other species, it is assumed that higher levels of biosecurity lead to a reduction in the need for antimicrobials and to improved animal health, thus increasing the welfare (Postma et al., 2017; Speksnijder et al., 2015). Biosecurity is the total of preventive measures to limit animals' and persons' exposure to disease agents (Anderson, 1998). External biosecurity measures are applied to limit the entrance of pathogens while internal biosecurity measures, often called biocontainment, help to control the spread of pathogens within the facility (Villarroel et al., 2007). A detailed read of biosecurity measures and application in dog kennels and breeding facilities can be found in *Biosecurity in animal production and veterinary medicine: From principles to practice* (Dendoncker et al., 2018).

Guidelines for biosecurity measures exist for group housing of dogs (Miller and Zawistowski, 2012) and some measures (e.g., duration of quarantine, use of non-porous materials, stocking density) are legally required (Code de l'Environnement, RD 14/08/1986, RD 27/04/2007, VLAREM). Unfortunately, to date, it is unclear if and how these guidelines are applied in canine breeding facilities, especially in small-scale dog breeders. Not all breeders seem to apply the same level of health management and biosecurity, likely because large-scale breeders have been associated with more disease in the past. In addition, prophylactic protocols may be insufficiently useful due to how they are applied, how breeders approach antimicrobial strategies, and how they approach general management (e.g., use of porous materials that are not easy to keep pathogen-free). Further examination of the above-mentioned issues can be achieved by mapping out existing health and biosecurity management in dog breeding.

Given the importance of disease prevention, the aim of this study was to investigate management, biosecurity practices and health protocols at different breeder types to be able to provide better guidance for future improvements.

3. MATERIALS AND METHODS

3.1. Study design

Given the protocol Directive 2010/63/EU did not apply and no permission from an Animal Ethics Committee had to be sought. A written informed consent was obtained from all dog breeders

before entry into this study. This study was designed as a stratified randomised cross-sectional study.

The target population consisted of persons breeding and selling dogs in Belgium, which included puppies bred locally as well as abroad. In Belgium, seven categories of dog breeders are legally defined (Table 4.1) and are regulated accordingly (RD 27/04/2007). This classification results in the appellation of the occasional breeder, the hobby breeder, the professional breeder and the breeder-merchant. This classification is based on the number of litters born yearly (less or equal than or more than ten) and further subdivision in small and large categories of breeders hinges on the number of females on site (less or equal than or more than ten), respectively. All breeders except occasional breeders are subject to accreditation before being allowed to breed and sell dogs. Based on these criteria, dog breeders producing more than two litters per year are registered and receive an accreditation. Legislation states that breeders may only merchandise self-bred puppies, with the exception of breeder-merchants, who may also supply puppies from a different source, e.g., puppies from other Belgian breeders or puppies originating from foreign countries.

The sampling frame consisted of a selection of accredited breeding facilities, listed by the government, and of all non-accredited breeders (producing less or equal than two litters per year) that could be identified through known sale channels such as: kennel club websites, canine breed clubs, specialised magazines, social networks, and online advertisements. The inclusion criteria for both accredited and non-accredited breeders were that the breeders were actively breeding or selling dogs, that at least one adult female dog was present on site, and that at least one puppy has been sold during the last two years before the visit (i.e., 2014 or 2015).

Randomisation of accredited dog breeders was achieved by stratified sampling from the registration database based on the Belgian classification, while aiming for an arbitrary number of breeders (n=15) for each stratum (Table 1). Randomisation of non-accredited breeders was achieved by performing a systematic random sampling (n=15) of dog breeders advertising a litter at that moment.

3.2. Data collection

All randomly selected breeders were contacted prior to an on-site visit to solicit their cooperation, as participation by breeders was voluntary. A response rate of 50.2% was obtained. The main reasons for participation refusal were: not actively breeding anymore, participation was deemed too time-consuming, retirement was imminent, or the investigator was unable to reach the breeder. Other, less common (<10%) reasons reported not to participate included: no particular interest in the study, change in regulations is not welcome, or the breeder and the investigator were not able to find a mutual schedule to plan a visit. Data were collected by the first author during an on-site semi-structured interview, using a check list on all aspects relevant to biosecurity and health management and observations at the site of the breeder. The check list was developed based upon factors described in literature as well as aspects described in the current legislations. More specifically, information was collected on breeding adults, pregnant dams, litters (both self-bred and acquired), ill animals, the structure of the facility and the materials used, husbandry practices, medical treatments and breeding performance. The 544 questions (of which 142 were open questions) were pretested and further optimised based on a preliminary visit to three breeding facilities of different categories. Selling numbers, frequencies of cleaning or medical treatments were reported by the breeder and not on sanitary documents, although observations prevailed reported information when both were available. All data were collected confidentially and coded anonymously in a spreadsheet (Microsoft Excel 2011®). Recruitment and data collection were performed from January to September 2016.

3.3. Data processing and statistical analysis.

Before analysis, skewed continuous data (i.e., dams on site, puppies born yearly) were normalised by means of a log10 transformation. Comparison of the breeder categories with regard to size (dams on site, adults on site, annual litters, yearly number of puppies sold) and implemented biosecurity measures was performed by one-way Analysis of Variance (ANOVA) for continuous variables (e.g., frequency of cleaning, number of animals) and Generalized Linear models were fitted to perform Binary Logistic regression for binomial variables (e.g., staff employment, disinfection). Pairwise comparisons of the data for each breeder categories with correction for multiple comparisons were performed respectively by Tukey post-hoc analysis for

continuous variables and by a generalised linear model with Šidák correction for binomial data. We provided the effect size as Eta Squared (η^2) and, the F-Value (F), degrees of freedom (dF) the statistical significance (corrected P-value) when possible for each continuous variable and we provided the effect size as mean difference (MD), corrected P-Value and Confidence Interval (CI) for each binomial variable. Significance level was set to 5%. All statistical analyses were done with SPSS Statistics v24 (IBM).

4. RESULTS

4.1. Breeder categories

Data were obtained from 102 Belgian dog breeders. Table 4.1 lists the distribution for each breeder category.

Table 4.1: Sampled dog breeder categories A to D compared to Belgian classification.

Dog breeders (category)	Small-scale breeders		Large-scale breeders		Total of Breeders*
	Occasional breeders	Occupational breeders	Commercial breeders	Merchants	
Occasional Breeder	14	2	0	0	unknown
Small Hobby Breeder	11	4	0	0	792
Large Hobby Breeder	7	8	0	0	82
Small Professional Breeder	4	11	0	0	33
Large Professional Breeder	2	11	2	0	116
Small Breeder-Merchant	4	5	0	4	47
Large Breeder-Merchant	0	4	5	4	37
Total of breeders visited	42	45	7	8	

*Number of breeders (n) registered in Belgium on 2015-11-30

Because 17.6% and 2.0% of the sampled breeders had more, respectively fewer dams on-site than was to be expected based on their accreditation and 10.8% produced more litters yearly, a great overlap was found between the Belgian categories. Therefore, it was decided to categorise the dog breeders into four categories (A, B, C, and D) based upon the number of

dams observed on site and the number of puppies reported to be sold per year instead of what they had once declared when filling in the accreditation forms for the government. A detailed description of our alternative dog breeder classification can be found in chapter 3. Table 4.2 depicts a comparison of the different breeder categories regarding the category defining variables (i.e., the observed number of dams on site and number of puppies sold per year as reported by the breeder).

Table 4.2: Number of animals (dams or puppies) at the different categories of breeders.

	Small-scale		Large-scale	
	Occasional	Occupational	Commercial	Merchant
DAMS ON-SITE				
Average number of dams on site (SD)	5.1[a] (2.5)	17.5[b] (8.7)	101.4[c] (51.1)	17.1[b] (17.3)
Median	5.0	16.0	87.0	9.0
Dams on site [min; max]	[1; 9]	[10; 34]	[56; 180]	[1; 47]
PUPPIES SOLD IN 2015				
Average number of puppies sold in 2015 (SD)	7.2[a] (6.1)	24.6[b] (28.1)	480.0[c] (403.5)	1092.9[c] (638.0)
Median	7.0	29.0	550.0	800.0
Puppies sold in 2015 [min; max]	[0; 21]	[0; 100]	[0; 1000]	[500; 2000]

[a-c] Index indicates a significant difference (P<0.05) between the means/percentages

4.2. General management at the different breeder categories

Of all breeders, 46.9% consisted of a family environment where under-aged children (<16 years) were involved in the daily husbandry practices. Adult breeding animals were mainly kept in pairs or in groups (94.8%), leaving individual housing less common among breeders. On average, pregnant females were isolated after 7.2 (standard deviation (SD) 1.1) weeks of pregnancy; no significant differences between breeder categories were found. Table 4.3 depicts a comparison of the different breeder categories regarding staff employment and additional size-related variables (i.e., the observed number of adult male dogs on site and the number of puppies born as reported by the breeder).

Table 4.3: Management at the different breeder categories.				
	Small-scale		Large-scale	
	Occasional	Occupational	Commercial	Merchant
Percentage of breeders to employ staff (%)	12.2[a]	14.3[a]	85.7[b]	85.7[b]
Average number of males on site (SD)	2.7[a] (2.0)	6.8[b] (5.4)	16.2[c] (3.8)	4.9[a,b] (4.1)
Average number of litters born in 2015 (SD)	2.5[a] (2.4)	8.0[a] (3.8)	126.0[b] (76.4)	10.7[a] (8.2)
Average number of puppies born in 2015 (SD)	11.7[a] (12.5)	54.5[a] (55.2)	532.2[b] (307.3)	120.0[a] (72.6)

[a-c] Index indicates a significant difference (P<0.05) between the means/percentage

4.3. External biosecurity measures at the different breeder types

A comparison of the different breeder categories regarding external biosecurity measures is depicted in tables 4.4 and 4.5. On average, breeders renewed 11.4% of the adult dogs annually. Limiting the acquisition of breeding dogs to only one supplier was recorded for 5.9% of breeders. More large-scale breeders quarantined (i.e., isolated for at least 48hours) newly acquired breeding dogs compared to small-scale breeders (MD=0.7; P<0.01; CI=0.5, 0.9), but all dog merchants reported to quarantine acquired puppies for at least 5 days (as legally defined) unless decided otherwise by the contracting veterinarian. Of all the breeders that declared to quarantine newly acquired dogs (n=25 of 102), adequate quarantine procedures (i.e., providing complete segregation in a dedicated room) were observed on-site in 72.0%. Only one breeder declared to quarantine dogs after exhibitions. Pest prevention (i.e., control of rodents, wildlife and birds) was found to be higher for commercial breeders compared to occasional breeders (MD=0.7; P<0.01; CI=0.3, 1.1) and occupational breeders (MD=0.6; P<0.01; CI= 0.2, 1.0).

Movements of dogs were defined by the number of times that a breeder declares to take his dog or her dog out of the facility (outward) in order to have contact with stranger dogs, or to accept stranger dogs into the breeding facility (inwards). Both inward (i.e., for boarding or mating) and outward (i.e., going to exhibitions, external mating) movements of dogs in general were requested for each breeding facility, as declared by the breeder. Movements of short

durations or in the immediate neighbourhood of the facility (i.e., going for a walk) were not considered.

An association was found between breeder type and the inward movements of strange dogs ($F_{3,98}$=5.6; P<0.01; η^2=0.15). Commercial breeders were found to accept dogs from external sources significantly more often than occasional breeders (MD=45.3; P<0.01; CI=15.8, 74.7), occupational breeders (MD=44.9; P<0.01; CI=15.6, 74.1) and than dog merchants (MD=41.3; P=0.02; CI=4.0, 78.6). No complete segregation of incoming strange dogs was recorded in 25 out of 36 breeders that declared to accept strange dogs. For these cases, we recorded shared rooms/outdoors, shared staff or shared equipment (i.e., without any sanitary measure in-between), and sometimes no segregation at all. While 33.3% of all breeders declared to perform outward movements with their dogs, an association was found between breeder type and outward movements ($F_{1,100}$=6.4; P=0.01; η^2=0.06). Small-scale breeders exposed their dogs significantly more (MD=8.0; P=0.01; CI=1.9, 14.2) to external contacts by outward movements than large-scale breeders. Overall, 2.0% of the visited breeders reported to limit the contact through mating by applying artificial insemination.

Non-regulated access of visitors to the maternity ward was significantly higher at small-scale breeders: occasional breeders regulated less access compared to commercial breeders (MD=0.5; P<0.01; CI=-0.8, -0.2) and merchants (MD=0.5; P<0.01; CI=-0.2, 0.8) and occupational breeders regulated less access compared to commercial breeders (MD=-0.6; P<0.01; CI=-0.9, -0.2) and merchants (MD=0.5; P=0.02; CI=0.1, 0.9). Non-regulated access of visitors to the nursery was also significantly higher at small-scale breeders compared to merchants: merchants regulated more access compared to occasional breeders (MD=0.4; P<0.01; CI=0.2, 0.6) and occupational breeders (MD=0.2; P=0.02; CI=0.0, 0.4).

Two percent of the visited breeders applied hygienic measures for vehicles (e.g., cleaning and disinfecting before entering or after leaving the facility, or restricting access to a dedicated path: a “dirty road”). Four breeders were familiar with the concept of clean and dirty road and all-in/all-out management; however, none of them applied the measures.

Table 4.4: External biosecurity measures applied by the different breeder categories.				
	Small-scale		Large-scale	
	Occasional	Occupational	Commercial	Merchant
GENERAL WIDELY APPLIED BIOSECURITY MEASURES (PERCENTAGE)				
Quarantine for acquired breeding dogs present	2.7%[a]	25.0%[b]	100.0%[c]	71.4%[c]
Pest control reported	17.1%[a]	24.4%[a]	85.7%[b]	50.0%[a,b]

[a-c] Index indicates a significant difference (P<0.05) between the means/percentages

Table 4.5: External biosecurity measures applied by the different breeder categories.				
	Small-scale		Large-scale	
	Occasional	Occupational	Commercial	Merchant
TRAFFIC OF ANIMALS TO OR FROM A BREEDING FACILITY				
Average number of adults acquired last 5 years (SD)	4.3[a] (3.7)	11.1[a] (8.6)[a]	77.9[b] (88.4)	32.0[a] (27.9)
Acquired dogs yearly / adult dogs on site	0.1[a] (0.1)	0.1[a] (0.06)	0.04[a] (0.01)	0.3[a] (0.02)
Average number of yearly outgoing movements	9.7[a] (13.3)	7.0[a] (9.9)	0.6[a] (1.5)	1.0[a] (2.7)
Average number of yearly incoming movements	0.4[a] (0.8)	0.9[a] (1.9)	45.7[b] (112.2)	5.0[a] (11.2)
Non-regulated access to adult dogs (% of access)	9.5[a]	9.2[a]	14.3[a]	14.3[a]
Non-regulated access to maternity ward (% of access)	21.5[a]	11.1[a]	0.0[b]	0.0[b]
Non-regulated access to nursery (% of access)	35.7[a]	15.6[a]	14.3[a,b]	0.0[b]

[a-b] Index indicates a significant difference (P<0.05) between the means/percentages

4.4. Internal biosecurity measures at the different breeder categories

Table 4.6, 4.7 and 4.8 depict a comparison of the breeder categories regarding internal biosecurity measures across the breeding facility. Visitation of the various infrastructures revealed a heterogeneity in the housing of dogs, ranging from private houses, garden sheds, outdoor kennels, old livestock stables, outdoor pens and more traditional pet-store setups with glass walls. No significant differences were found in the choice of flooring (e.g., grass, concrete, gravel, etc.) for the outdoor areas.

All visited breeders applied the following physical compartmentalisation at their facility: adult dogs (for breeding males and females that are not yet isolated if pregnant or not kept with puppies anymore, table 4.6), maternity ward (when pregnant dams are separated from the adults for whelping and stay there with puppies until weaning, table 4.7) and nursery (only for puppies that are not sold yet, table 4.8). The presence of a sick-bay, allowing the segregation of diseased animals, was mainly observed in large-scale breeders: a sick bay was observed more often at commercial breeders compared to occasional breeders (MD=0.7; P<0.01; CI=0.2, 1.1) and occupational breeders (MD=0.7; P< 0.01; CI=0.2, 1.1) and more often at merchants compared to occasional breeders (MD= 0.5; P=0.05; CI=0.0, 1.0). Retired dogs (dogs without a breeding purpose, i.e., retired dams and sires) were kept by 47.1% of all breeders. All commercial breeders declared to keep retired dogs. Additional compartmentalisations recorded were: a quarantine area (observed in 18.0% of all breeders) and a showroom (observed in 13.7% of all breeders). In a showroom, breeders can present the pups that are born on-site or, in the case of merchants, originate from other breeders to potential owners. No facility included a hygiene lock, a separate room for applying hygienic measures such as changing clothes and footwear before entering a compartment of the animal facilities.

The presence of porous materials (i.e., uncoated softwood and hardboard, natural stone, unsealed concrete, soil) was assessed in each compartment, and also for the whelping box (a confined space where the dam is kept during whelping and nursing). Porous materials were least the most used by occasional breeders in the compartment of the adult dogs compared to merchants (MD=0.2; P=0.05; CI=0.0, 0.5) and in the nursery compared to commercial breeders (MD=0.3; P=0.05; CI=0.0, 0.5).

Overall, ventilation and humidity were mechanically regulated in 12.5% and 3.9% of the breeding facilities respectively. With regard to cleaning of the facility (i.e., removing waste and medium) both dry cleaning, on average 20.6 times a week (SD 11.0), and wet cleaning on average 6.6 (SD 4.8) times a week, were applied broadly across all breeder categories. With regard to disinfection (i.e., killing most of pathogens) 31.4% of all breeders applied disinfection preceded by cleaning regularly in the maternity ward and in the nursery. Disinfection across all compartments was applied most by commercial breeders and merchants. Commercial breeders declared more often to perform disinfection at the adult dogs compared to occasional

breeders (MD=0.5; P=0.02; CI=0.1, 1.0) and in the nursery compared to occasional (MD=0.7; P<0.01; CI=0.3, 1.1) and occupational breeders (MD=0.6; P<0.01; CI=0.2, 1.0). Merchants declared more often to perform disinfection more often than occasional breeders and occupational breeders at the adult dogs (MD=0.7; P<0.01; CI=0.4, 1.1 and MD=0.6; P<0.01; CI=0.2, 0.9 respectively), at the maternity ward (MD=0.7; P<0.01; CI=0.3, 1.1 and MD=0.6; P<0.01; CI=0.2, 1.0 respectively) and at the nursery (MD=0.7; P<0.01; CI=0.4, 1.1 and MD=0.6; P<0.01; CI=0.2, 0.9 respectively).

While investigating the disinfectants used, 19 breeders stated that they were not confident with their choice or would like guidelines or a course to gain knowledge. The main reasons given to choose a disinfectant were: previous experiences, recommended by another breeder, or readily accessible (i.e., sodium hypochlorite solution). No significant differences between type of breeders were found in providing outside access to adult dogs, although differences appeared when considering if outdoor access was free or on the opposite regulated by the caretaker (e.g., only fixed hours every day or specific days of the week).

Table 4.6: Internal biosecurity measures applied by the different dog breeder categories.

	Small-scale		Large-scale	
	Occasional	Occupational	Commercial	Merchant
PRESENCE OF OTHER DOGS ON SITE (PERCENTAGE)				
Presence of retired dogs	50.0[a]	46.7[a]	100.0[b]	37.5[a]
Segregation of diseased animals	19.0[a]	25.6[a]	100.0[b]	87.5[b]
APPLICATION OF INTERNAL BIOSECURITY MEASURES AT ADULT DOGS (PERCENTAGE)				
Porous materials absent	19.0[a]	27.3[a,b]	28.6[a,b]	62.5[b]
Temperature control present	77.5[a]	50.0[b]	42.9[a,b,c]	28.6[c]
Ventilation control present	12.2[a]	2.4[a]	14.3[a]	14.3[a]
Disinfection monthly	17.1[a]	28.9[a]	71.4[b]	87.5[b]
Regulated access to outside	2.4[a]	15.6[b]	28.6[a,b]	0.00[a]
Outdoors cleanable	17.1[a]	15.8[a]	60.0[a,b]	62.5[b]
WEEKLY AVERAGE OF CLEANING AT ADULT DOGS				
Dry cleaning weekly (SD)	6.1[a] (3.5)	6.9[a] (4.1)	4.3[a] (2.0)	5.7[a] (2.2)
Wet cleaning weekly (SD)	2.0[a] (1.6)	2.4[a] (1.9)	1.2[a] (0.6)	2.7[a] (2.0)

[a-c] Index indicates a significant difference (P<0.05) between the means/percentages

Table 4.7: Internal biosecurity measures applied by the different dog breeder categories.				
	Small-scale		Large-scale	
	Occasional	Occupational	Commercial	Merchant
APPLICATION OF INTERNAL BIOSECURITY MEASURES AT MATERNITY WARD (PERCENTAGE)				
Porous materials absent	40.5[a]	53.3[a]	71.4[a]	62.5[a]
Whelping box non-porous	39.4[a]	37.1[a]	66.7[a]	28.6[a]
Temperature control present	92.5[a]	59.5[b]	57.1[a,b]	50.0[b]
Ventilation control present	15.4[a]	0.0[b]	14.3[a,b]	12.5[a,b]
Disinfection when empty	20.0[a]	31.0[a]	57.1[a,b]	87.5[b]
Regulated access to outside	12.2[a]	15.6[a]	71.4[b]	62.5[b]
Outdoors cleanable	16.7[a]	21.1[a]	100.0[b]	33.3[a]
WEEKLY AVERAGE OF CLEANING AT MATERNITY WARD				
Dry cleaning weekly (SD)	6.6[a] (4.4)	5.9[a] (4.2)	5.6[a] (2.4)	4.4[a] (2.2)
Wet cleaning weekly (SD)	2.1[a] (1.9)	2.2[a] (1.8)	1.3[a] (0.5)	2.4[a] (2.2)

[a-b] Index indicates a significant difference (P<0.05) between the means/percentages

Table 4.8: Internal biosecurity measures applied by the different dog breeder categories.				
	Small-scale		Large-scale	
	Occasional	Occupational	Commercial	Merchant
APPLICATION OF INTERNAL BIOSECURITY MEASURES AT NURSERY (PERCENTAGE)				
Separate room	17.5[a]	80.0[b]	100.0[c]	100.0[c]
Porous materials absent	33.3[a]	53.3[a,b]	71.4[b]	62.5[a,b]
Temperature control	90.5[a]	64.4[b]	42.9[a,b,c]	25.0[c]
Ventilation control	11.9[a]	2.2[a]	14.3[a]	12.5[a]
Disinfection when empty	14.3[a]	28.9[a]	14.3[b]	12.5[b]
Regulated access to outside	9.5[a]	15.6[a]	66.7[b]	71.4[b]
Outdoors cleanable	12.5[a]	20.5[a]	100.0[b]	40.0[a]
WEEKLY AVERAGE OF CLEANING AT NURSERY				
Dry cleaning weekly (SD)	9.9[a] (8.1)	7.7[a] (5.8)	6.1[a] (1.9)	6.3[a] (1.9)
Wet cleaning weekly (SD)	2.4[a] (2.0)	2.4[a] (2.1)	1.5[a] (0.7)	2.6[a] (2.1)

[a-c] Index indicates a significant difference (P<0.05) between the means/percentages

Table 4.9 lists the reported hygienic measures for each breeder category applied at the different compartments (the adult dogs, the maternity ward and the nursery). Systematically applied hygienic measures included measures that the breeders declared to always perform, such as

hand hygiene (i.e., washing hands, wearing gloves), footwear hygiene (i.e., cleaning shoes, wearing boot covers, using dedicated shoes), and the wear of dedicated clothing (wearing clothes specific to a compartment or wearing a disposable coverall).

Over all compartments, most hygienic measures were applied by large-scale breeders. Compared to small-scale breeders they reported to apply hygienic measures more often at the maternity ward (MD=0.4; P<0.01; CI=0.1, 0.7) and the nursery (MD=0.3; P=0.04; CI=0.0, 0.5) and had a tendency to apply hygienic measures more often at the adult dogs (MD=0.2; P=0.08; CI=0.0, 0.5). The use of porous materials was associated with the absence of systematically applied hygienic measures at the maternity ward (MD=0.3; P<0.01; CI=0.1, 0.4) and there was a tendency at the nursery (MD=0.1; P=0.1; CI=0.0, 0.2). The type of hygienic measures applied was not significantly different between breeder categories. The use of porous materials was also associated with the absence of disinfection at the maternity ward (MD=0.2; P=0.02; CI=0.0, 0.4) and at the nursery (MD=0.4; P<0.01; CI=0.2, 0.5) and there was a tendency at the adult dogs (MD=0.2; P=0.06; CI=0.0, 0.4).

Table 4.9: Systematic application of hygienic measures according to breeder category.

		Small-scale	Large-scale
APPLICATION OF HYGIENIC MEASURES AT ADULT DOGS (PERCENTAGE OF BREEDERS)			
Yes		12.3[a]	35.7[a]
Of which:	hand hygiene	60.2[a]	0.0[a]
	hand and footwear hygiene	39.8[a]	80.1[a]
	hand and footwear hygiene and dedicated clothing	0.0[a]	19.9[a]
APPLICATION OF HYGIENIC MEASURES AT MATERINITY (PERCENTAGE OF BREEDERS)			
Yes		20.7[a]	60.0[b]
Of which:	hand hygiene	27.5[a]	33.3[a]
	hand and footwear hygiene	60.9[a]	44.5[a]
	hand and footwear hygiene and dedicated clothing	11.1[a]	22.2[a]
APPLICATION OF HYGIENIC MEASURES AT NURSERY (PERCENTAGE OF BREEDERS)			
Yes		8.0[a]	33.3[b]
Of which:	hand hygiene	57.5[a]	20.1[a]
	hand and footwear hygiene	42.5[a]	40.0[a]
	hand and footwear hygiene and dedicated clothing	0.0[a]	40.0[a]

[a-b] Index indicates a significant difference (P<0.05) between the percentages

4.5. Medical treatments at the different breeder categories

Prophylactic treatments such as vaccination and endoparasite control were widely adopted across all categories (Table 4.10). Out of all sampled breeders, 13.8% responded that they systematically administered antimicrobials to females after parturition (in the maternity ward) and 10.3% of them responded that they systematically administered antimicrobials to all their puppies, or at least to puppies of one breed (in the nursery). Of all the identified types of antimicrobials in the maternity ward (for females after parturition) (n=10), the most commonly used were amoxicilin-clavulinic acid (n=6), followed by trimethoprim-sulphonamid (n=2) and amoxicillin (n=2). Of all the identified types of antimicrobials in the nursery (for puppies) (n=8), the most commonly used are metronidazole-spiramycin (n=6), erythromycin (n=1), and amoxicillin-clavulanic acid (n=1). The metronidazole-spiramycin formulation (Stomorgyl®, Merial) was systematically administered (the breeder declared to always treat) in response to intermittent intestinal signs. Underlying giardiasis was assumed based on similarities with previous outbreaks, but was not individually tested through microscopic examination or laboratory findings.

Table 4.10: Preventive medical treatment as reported by dog breeders.

	Small-scale		Large-scale	
	Occasional	Occupational	Commercial	Merchant
MEDICAL TREATMENTS (PERCENTAGE OF BREEDERS)				
Vaccination adult dogs	85.4^{a}	92.7^{a,b}	100.0^{b}	100.0^{b}
Vaccination puppies	95.2^{a}	100.0^{a}	100.0^{a}	100.0^{a}
Endoparasite control of adult dogs	85.4^{a}	95.1^{a,b}	100.0^{b}	100.0^{b}
Endoparasite control of puppies	95.2^{a}	100.0^{a}	100.0^{a}	100.0^{a}
Systematic antimicrobial treatment of adult dogs	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
Systematic antimicrobial treatment in maternity ward (dams)	4.8^{a}	24.4^{b}	14.3^{a,b}	12.5^{a,b}
Systematic antimicrobial treatment in nursery (puppies)	4.9^{a,b}	9.5^{a}	14.3^{a,b}	0.0^{b}
Ectoparasitic treatment of adult dogs	41.5^{a}	48.8^{a}	28.6^{a}	28.6^{a}

$^{a-b}$ Index indicates a significant difference ($P<0.05$) between the means/percentage

5. DISCUSSION

To the best of our knowledge, this is the first study investigating the facility management, the applied biosecurity measures and the prophylactic protocols in different categories of dog breeding facilities.

Stratified sampling provided an overview of all breeder categories. Although selection bias in our study was limited by random sampling of the accredited breeders, it remains noteworthy that participation was based on willingness to collaborate. Because of the acceptable response rate of breeders and the main reasons given not to participate, a limited enrollment bias is expected. Not all data could be verified on-site, for example how often a breeder provides outdoor access, the cleaning frequency or the systemic administration of therapeutics. Also, the applied biosecurity measures were not put into relation to the disease incidence or mortality, and more research including measuring these parameters would enable some more firm conclusions to be reached. Nonetheless, we believe that the collected information provides a good insight into the level of application of biosecurity measures in the different dog breeder categories. A general approach was chosen in order to assess all factors possibly influencing the biosecurity in dog breeding facilities without compromising time and financial limitations. More in-depth research of specific measures would be deemed profitable.

Considering the fact that larger breeding facilities are more likely to face disease outbreaks (Schumaker et al., 2012; Zicola et al., 2012), consistent and correct implementation of biosecurity measures should be a fundamental element of large-scale breeder management. More external interaction and sources result in a higher risk of introduction of pathogens. For instance, acquisition of new animals can result in the introduction of pathogens into the breeding facility (Villarroel et al., 2007). The interview with the breeders revealed anecdotal reports of a “20% renewing rule”, meaning that a breeder replaces 20% of breeding dogs every year. While a handful of breeders stuck to this empirical rule, lower figures were reported for most breeder categories.

Historically, quarantine has been an effective method to limit the introduction of diseases in a confined area by performing the segregation of animals (Gensini et al., 2004). The importance of quarantine in canine facilities has also been recognised by several authors (ESCCAP, 2010b;

Hubrecht et al., 1992; Loveridge, 1998; Simmons and Hoffman, 2016). Quarantine limits the introduction of disease through direct transmission; however, indirect transmission should not be overlooked. Visitors, rodents, birds, wildlife, vehicles, food and water are, in analogy to other species (Gelaude et al., 2014; Laanen et al., 2013), potential canine disease transmitters (Crawford et al., 2005; Dobler and Pfeffer, 2011; LeJeune and Hancock, 2001; Song et al., 2008; Umhang et al., 2014). In this study, implementation of quarantine, a compulsory measure for all merchants when gathering and selling dogs of different origins, was significantly higher in large-scale breeders. The legislative minimum duration of quarantine of five days, however, does not cover the incubation period for several important pathogens such as *Bordetella bronchiseptica* and Canine Parainfluenzavirus (CPIV) (4-6 days), or CPV (3-7 days), Canine Infectious Hepatitis (CIH) (4-9 days), and Canine Herpesvirus (CHV) (6-10 days) (Decaro et al., 2008; Lamm and Rezabek, 2008; Miranda and Thompson, 2016; Thrusfield et al., 1991). Arguably, it would be better to implement quarantine for a minimum duration of the longest incubation period of the most common pathogen, resulting in a minimal advised duration of 10 days. However, when it concerns young dogs, the psychological well-being and socialisation of the animal might be compromised during the segregation, and should be taken into account.

This study showed that the inward movements (i.e., purchase, exchange, and boarding) of puppies and adult dogs is a common practice at most dog breeders. Additionally, the number of breeders that reported to board external dogs (i.e., for mating purposes or as a boarding service) was surprisingly high. Since boarding kennels are a challenging environment for biocontainment (Buonavoglia and Martella, 2007; Ronsse et al., 2002), these circumstances could facilitate the spread of pathogens to the breeding facility and should therefore always be avoided, especially by commercial breeders.

The number of outward movements of dogs was reasonably high in the breeder sample, with some occasional breeders having over 40 outward movements per year for one or more dogs. These numbers were partly caused by external mating (33.3% of all breeders), and were also expected to be relatively high since the importance of increasing the genetic diversity in bloodlines by outbreeding has been established (Leroy, 2011). A second explanation is the large number of exhibitions that dogs are required to participate in order to be allowed in the book of origins. High rates of outward movement, however, should be considered a risk for pathogen

transmission, especially during canine exhibitions, since a large number of dogs and visitors from different origins are gathered. Outbreaks in canine exhibitions have been reported (Primm, 2017), resulting from both direct transmission through the dogs as well as indirect transmission through visitors, who often share intense contact with other canine populations (Bender et al., 2004; Grgič-Vitek et al., 2015).

Prevention of disease can be further achieved through cleaning and disinfection. Physical cleaning (dry and wet) is the removal of waste and organic materials. Cleaning does not kill pathogens but removes most of the medium. Disinfection is the process of reducing the number of pathogens (Gilman, 2004). Protocols including dry and wet cleaning were largely implemented, especially the regular removal of organic material; however, improvements can be made considering disinfection. In small-scale breeders, the use and knowledge of disinfection were relatively moderate. The choice of disinfectant products and their application was based on habits or empirical knowledge. Since disinfection is the keystone for control of environmentally resistant pathogens (Fiechter et al., 2012), improvement in the education of dog breeders and implementation of category-adjusted hygiene guidelines could increase the awareness and application of disinfection across all dog breeder categories.

The periparturient period has been proven as the period with the highest risk of morbidity and mortality for puppies and dams, and particular hygiene should be introduced when isolating the dams and during the first weeks of the puppies' lives (Evermann and Wills, 2011; Lloyd et al., 1983). Although measures are more often applied in large-scale breeders, a substantial number of breeders across all categories could improve the periparturient hygiene. The relatively frequent use of porous materials in the maternity ward (in particular for the whelping box) reveals an absence of knowledge in the cleaning and disinfection of surfaces rather than an absence of awareness in the importance of it. Porous materials were least used in the maternity ward by the dog breeders who applied most hygienic measures in the maternity ward and performed disinfection more frequently. This may suggest that breeders who are more aware of biosecurity are also better informed about the choice of surfaces.

The data recorded on antimicrobial use are in agreement with earlier reports of misuse of antimicrobials in Italian canine breeding facilities (Rota et al., 2013, 2011). Moreover, these data might well be an underestimate as they are based on self-reporting and no official registration

exists. Resistance to broader spectrum antimicrobials (i.e., amoxicillin-clavulanic acid) in *Escherichia coli* (*E. coli*) isolates (Normand et al., 2000) is a phenomenon that could be partially explained by excessive and incorrect implementation of this drug. This is worrying since shedding of *E. coli* by the pregnant bitch increases during the periparturient period (Evermann and Wills, 2011) and has been reported as the most frequent isolate in postpartum mortality of puppies (Meloni et al., 2014; Nielen et al., 1998).

This study supports the suspicion that systemic administration of antimicrobials is regularly based on assumptions, earlier experiences, or empirical knowledge, rather than on clinical diagnoses.

6. CONCLUSION

Data collected during this study demonstrated that large-scale breeders are associated with a higher implementation of biosecurity measures. However, substantial improvements of internal and external biosecurity measures are desirable in all categories. To be efficacious and feasible, these measures should be tailored to the different categories of breeders. The characterisation of the different types of dog breeders and their biosecurity and management practices in this study are a first step towards tailored recommendations, but the relative paucity in scientific literature calls for more research in the field of management of canine facilities, with the emphasis on prevention of introduction and spread of disease, while taking into account behavioural development and wellbeing of breeding stock puppies to be sold.

7. REFERENCES

Anderson, J.F., 1998. Biosecurity: A new term for an old concept: How to apply it. Bov. Pract. 32, 61–70.

Australian Government - Department of Agriculture, 2013. Importation of dogs and cats and their semen from approved countries.

AVMA, 2012. U.S. pet ownership and demographics sourcebook. American Veterinary Medical Association, Schaumburg, IL.

Bandai, C., Ishiguro, S., Masuya, N., Hohdatsu, T., Mochizuki, M., 1999. Canine coronavirus infections in Japan: virological and epidemiological aspects. J. Vet. 61, 731–736.

Beerda, B., Schilder, M.B.., Bernadina, W., Van Hooff, J.A.R.A.., De Vries, H.W., Mol, J.A., 1999. Chronic Stress in Dogs Subjected to Social and Spatial Restriction. II. Hormonal and Immunological Responses. Physiol. Behav. 66, 243–254. https://doi.org/10.1016/S0031-9384(98)00290-X

Beerda, B., Schilder, M.B.H., van Hooff, J.A.R.A.M., de Vries, H.W., 1997. Manifestations of chronic and acute stress in dogs. Appl. Anim. Behav. Sci. 52, 307–319. https://doi.org/10.1016/S0168-1591(96)01131-8

Bender, J.B., Shulman, S.A., Veterinarians, A. in P.C. subcommittee of the N.A. of S.P.H., 2004. Reports of zoonotic disease outbreaks associated with animal exhibits and availability of recommendations for preventing zoonotic disease transmission from animals to people in such settings. J. Am. Vet. Med. Assoc. 224, 1105–1109. https://doi.org/10.2460/javma.2004.224.1105

Beugnet, F., Marié, J.-L., 2009. Emerging arthropod-borne diseases of companion animals in Europe. Vet. Parasitol. 163, 298–305. https://doi.org/10.1016/j.vetpar.2009.03.028

Bohm, J., Blixenkrone-Møller, M., Lund, E., 1989. A serious outbreak of canine distemper among sled-dogs in northern Greenland. Arctic Med. Res. 48, 195–203.

Bramble, M., Morris, D., Tolomeo, P., Lautenbach, E., 2011. Potential Role of Pet Animals in Household Transmission of Methicillin-Resistant *Staphylococcus aureus:* A Narrative Review. Vector-Borne Zoonotic Dis. 11, 617–620. https://doi.org/10.1089/vbz.2010.0025

Bugg, R.J., Robertson, I.D., Elliot, A.D., Thompson, R.C.A., 1999. Gastrointestinal Parasites of Urban Dogs in Perth, Western Australia. Vet. J. 157, 295–301. https://doi.org/10.1053/tvjl.1998.0327

Buonavoglia, C., Martella, V., 2007. Canine respiratory viruses. Vet. Res. 38, 355–373. https://doi.org/10.1051/vetres:2006058

Clarke, A.S., Wittwer, D.J., Abbott, D.H., Schneider, M.L., 1994. Long-term effects of prenatal stress on HPA axis activity in juvenile rhesus monkeys. Dev. Psychobiol. 27, 257–269. https://doi.org/10.1002/dev.420270502

Cliquet, F., Verdier, Y., Sagné, L., Aubert, M., 2003. titration in 25,000 sera of dogs and cats vaccinated against rabies in France, in the framework of the new regulations that offer an alternative to quarantine. Rev. Sci.

Crawford, P., Dubovi, E., Castleman, W., 2005. Transmission of equine influenza virus to dogs. Science (80-.). 310, 482–485.

Dahlöf, L.-G., Hård, E., Larsson, K., 1978. Influence of maternal stress on the development of the fetal genital system. Physiol. Behav. 20, 193–195.

Davis-Wurzler, G.M., 2014. 2013 update on current vaccination strategies in puppies and kittens. Vet Clin North Am Small Anim Pr. 44, 235–263. https://doi.org/10.1016/j.cvsm.2013.11.006

Day, M.J., 2007. Immune System Development in the Dog and Cat. J. Comp. Pathol. 137, S10–S15. https://doi.org/10.1016/j.jcpa.2007.04.005

Day, M.J., Horzinek, M.C., Schultz, R.D., 2007. Guidelines for the vaccination of dogs and cats. Compiled by the Vaccination Guidelines Group (VGG) of the World Small Animal Veterinary Association (WSAVA). J. Small Anim. Pract. 48, 528–541. https://doi.org/10.1111/j.1748-5827.2007.00462.x

Day, M.J., Horzinek, M.C., Schultz, R.D., Squires, R.A., 2016. WSAVA Guidelines for the vaccination of dogs and cats [WWW Document]. J. Small Anim. Pract. https://doi.org/10.1111/jsap.2_12431

De Graef, E.M., Decostere, A., Devriese, L.A., Haesebrouck, F., 2004. Antibiotic Resistance among Fecal Indicator Bacteria from Healthy Individually Owned and Kennel Dogs. Microb. Drug Resist. 10, 65–69. https://doi.org/10.1089/107662904323047826

Decaro, N., Buonavoglia, C., 2012. Canine parvovirus—A review of epidemiological and diagnostic aspects, with emphasis on type 2c. Vet. Microbiol. 155, 1–12. https://doi.org/10.1016/j.vetmic.2011.09.007

Decaro, N., Martella, V., Buonavoglia, C., 2008. Canine Adenoviruses and Herpesvirus. Vet. Clin. Small Anim. Pract. 38, 799–814. https://doi.org/10.1016/j.cvsm.2008.02.006

Dendoncker, P.-A., de Rooster, H., Abma, E., Wydooghe, E., Dewulf, J., 2018. Biosecurity measures for dog merchants and canine breeding kennels, in: Dewulf, J., Van Immerseel, F. (Eds.), Biosecurity in Animal Production and Veterinary Medicine : From Principles to Practice. Acco, Leuven / Den Haag, pp. 433–452.

Dobler, G., Pfeffer, M., 2011. Fleas as parasites of the family Canidae. Parasit. Vectors 4, 139. https://doi.org/10.1186/1756-3305-4-139

Dotson, M.J., Hyatt, E.M., 2008. Understanding dog–human companionship. J. Bus. Res. 61, 457–466. https://doi.org/10.1016/j.jbusres.2007.07.019

Dupont, S., Butaye, P., Claerebout, E., Theuns, S., Duchateau, L., Van de Maele, I., Daminet, S., 2013. Enteropathogens in pups from pet shops and breeding facilities. J. Small Anim. Pract. 54, 475–480. https://doi.org/10.1111/jsap.12119

Ellis, W.A., 2010. Control of canine leptospirosis in Europe: time for a change? Vet. Rec. 167, 602–605. https://doi.org/10.1136/vr.c4965

ESCCAP, 2010a. Control of Intestinal protozoa in Dogs and Cats - ESCCAP Guidelines 2nd edition [WWW Document]. URL https://www.esccap.org/guidelines/

ESCCAP, 2010b. Worm Control in Dogs and Cats - ESCCAP Guidelines 2nd edition [WWW Document]. URL https://www.esccap.org/guidelines/

European Commission - Food Safety, 2017. Imports from non-EU countries [WWW Document]. URL https://ec.europa.eu/food/animals/pet-movement/eu-legislation/non-eu-imports_en (accessed 8.14.17).

Evermann, J.F., Wills, T.B., 2011. Immunologic development and immunization, in: Peterson, M.E., Kutzler, M.A. (Eds.), Small Animal Pediatrics: The First 12 Months of Life. Saunders/Elsevier, St Louis, pp. 104–112. https://doi.org/https://doi.org/10.1016/C2009-0-37825-3

Fiechter, R., Deplazes, P., Schnyder, M., 2012. Control of Giardia infections with ronidazole and intensive hygiene management in a dog kennel. Vet. Parasitol. 187, 93–98. https://doi.org/10.1016/j.vetpar.2011.12.023

Finley, R., Reid-Smith, R., Ribble, C., Popa, M., Vandermeer, M., Aramini, J., 2008. The Occurrence and Antimicrobial Susceptibility of Salmonellae Isolated from Commercially Available Canine Raw Food Diets in Three Canadian Cities. Zoonoses Public Health 55, 462–469. https://doi.org/10.1111/j.1863-2378.2008.01147.x

Fox, M.W., Stelzner, D., 1966. Behavioural effects of differential early experience in the dog. Anim. Behav. 14, 273–281. https://doi.org/10.1016/S0003-3472(66)80083-0

Gelaude, P., Schlepers, M., Verlinden, M., Laanen, M., Dewulf, J., 2014. Biocheck.UGent: A quantitative tool to measure biosecurity at broiler farms and the relationship with technical performances and antimicrobial use. Poult. Sci. 93, 2740–2751. https://doi.org/10.3382/ps.2014-04002

Gensini, G., Yacoub, M., Conti, A., 2004. The concept of quarantine in history: from plague to SARS. J. Infect. 49, 257–261.

Ghneim, G.S., Viers, J.H., Chomel, B.B., Kass, P.H., Descollonges, D.A., Johnson, M.L., 2007. Use of a case-control study and geographic information systems to determine environmental and demographic risk factors for canine leptospirosis. Vet. Res. 38, 37–50. https://doi.org/10.1051/vetres:2006043

Gilman, N., 2004. Sanitation in the animal shelter, in: Miller, L., Zawistowski, S. (Eds.), Shelter Medicine for Veterinarians and Staff. Blackwell Publishing, Ames, Iowa, pp. 67–78.

Glickman, L.T., Shofer, F.S., 1987. Zoonotic Visceral and Ocular Larva Migrans. Vet. Clin. North Am. Small Anim. Pract. 17, 39–53. https://doi.org/10.1016/S0195-5616(87)50604-0

Grgič-Vitek, M., Frelih, T., Ucakar, V., Fafangel, M., Markocic, O.J., Prosenc, K., Kraigher, A., 2015. An outbreak of measles associated with an international dog show in Slovenia, November 2014. Eurosurveillance 20, 21012.

Hird, D., Ruble, R., Reagor, Sg.D.D. et al 1992. pdfFARE/Hir. et al 1992. pd., 1992. Morbidity and mortality in pups from pet stores and private sources: 968 cases (1987-1988). J. Am. Vet. Med. Assoc. 201, 471–474.

Hollett, R.B., 2006. Canine brucellosis: Outbreaks and compliance. Theriogenology 66, 575–587. https://doi.org/10.1016/j.theriogenology.2006.04.011

Hubrecht, R.C., Serpell, J.A., Poole, T.B., 1992. Correlates of pen size and housing conditions on the behaviour of kennelled dogs. Appl. Anim. Behav. Sci. 34, 365–383. https://doi.org/10.1016/S0168-1591(05)80096-6

Jansen, A., Schöneberg, I., Frank, C., Alpers, K., Schneider, T., Stark, K., 2005. Leptospirosis in Germany, 1962-2003. Emerg. Infect. Dis. 11, 1048–54. https://doi.org/10.3201/eid1107.041172

Jones, J.L., Dargelas, V., Roberts, J., Press, C., Remington, J.S., Montoya, J.G., 2009. Risk Factors for *Toxoplasma gondii* Infection in the United States. Clin. Infect. Dis. 49, 878–884. https://doi.org/10.1086/605433

Kapel, C.M.O., Torgerson, P.R., Thompson, R.C.A., Deplazes, P., 2006. Reproductive potential of Echinococcus multilocularis in experimentally infected foxes, dogs, raccoon dogs and cats. Int. J. Parasitol. 36, 79–86. https://doi.org/10.1016/j.ijpara.2005.08.012

King, T., Marston, L.C., Bennett, P.C., 2012. Breeding dogs for beauty and behaviour: Why scientists need to do more to develop valid and reliable behaviour assessments for dogs kept as companions. Appl. Anim. Behav. Sci. 137, 1–12. https://doi.org/10.1016/J.APPLANIM.2011.11.016

Laanen, M., Persoons, D., Ribbens, S., de Jong, E., Callens, B., Strubbe, M., Maes, D., Dewulf, J., 2013. Relationship between biosecurity and production/antimicrobial treatment characteristics in pig herds. Vet. J. 198, 508–512. https://doi.org/10.1016/j.tvjl.2013.08.029

Lamm, C.G., Rezabek, G.B., 2008. Parvovirus Infection in Domestic Companion Animals. Vet. Clin. Small Anim. Pract. 38, 837–850. https://doi.org/10.1016/j.cvsm.2008.03.008

Larson, G., Karlsson, E.K., Perri, A., Webster, M.T., Ho, S.Y.W., Peters, J., Stahl, P.W., Piper, P.J., Lingaas, F., Fredholm, M., Comstock, K.E., Modiano, J.F., Schelling, C., Agoulnik, A.I., Leegwater, P.A., Dobney, K., Vigne, J.-D., Vila, C., Andersson, L., Lindblad-Toh, K., 2012. Rethinking dog domestication by integrating genetics, archeology, and biogeography. Proc. Natl. Acad. Sci. 109, 8878–8883. https://doi.org/10.1073/pnas.1203005109

Lawler, D.F., 2008. Neonatal and pediatric care of the puppy and kitten. Theriogenology 70, 384–392. https://doi.org/10.1016/j.theriogenology.2008.04.019

Lee, P.R., Brady, D.L., Shapiro, R.A., Dorsa, D.M., Koenig, J.I., 2007. Prenatal stress generates deficits in rat social behavior: Reversal by oxytocin. Brain Res. 1156, 152–167. https://doi.org/10.1016/j.brainres.2007.04.042

LeJeune, J.T., Hancock, D.D., 2001. Public health concerns associated with feeding raw meat diets to dogs. J. Am. Vet. Med. Assoc. 219, 1222–1225. https://doi.org/10.2460/javma.2001.219.1222

Leroy, G., 2011. Genetic diversity, inbreeding and breeding practices in dogs: Results from pedigree analyses. Vet. J. 189, 177–182. https://doi.org/10.1016/j.tvjl.2011.06.016

Lindblad-Toh, K., Wade, C.M., Mikkelsen, T.S., Karlsson, E.K., Jaffe, D.B., Kamal, M., Clamp, M., Chang, J.L., Kulbokas, E.J., Zody, M.C., 2005. Genome sequence, comparative analysis and haplotype structure of the domestic dog. Nature 438, 803–819. https://doi.org/10.1038/nature04338

Lloyd, S., Amerasinghe, P.H., Soulsby, E.J.L., 1983. Periparturient immunosuppression in the bitch and its influence on infection with Toxocara canis. J. Small Anim. Pract. 24, 237–247. https://doi.org/10.1111/j.1748-5827.1983.tb00437.x

Looney, A.L., Bohling, M.W., Bushby, P.A., Howe, L.M., Griffin, B., Levy, J.K., Eddlestone, S.M., Weedon, J.R., Appel, L.D., Rigdon-Brestle, Y.K., Ferguson, N.J., Sweeney, D.J., Tyson, K.A., Voors, A.H., White, S.C., Wilford, C.L., Farrell, K.A., Jefferson, E.P., Moyer, M.R., Newbury, S.P., Saxton, M.A., Scarlett, J.M., 2008. The Association of Shelter Veterinarians veterinary medical care guidelines for spay-neuter programs. J. Am. Vet. Med. Assoc. 233, 74–86. https://doi.org/10.2460/javma.233.1.74

Loveridge, G.., 1998. Environmentally enriched dog housing. Appl. Anim. Behav. Sci. 59, 101–113. https://doi.org/10.1016/S0168-1591(98)00125-7

Lye, D.J., 2002. Health risks associated with consumption of untreated water from household roof catchment systems. J. Am. Water Resour. Assoc. 38, 1301–1306. https://doi.org/10.1111/j.1752-1688.2002.tb04349.x

McDonnell, G., Russell, A.D., 1999. Antiseptics and disinfectants: activity, action, and resistance. Clin. Microbiol. Rev. 12, 147–79.

Meloni, T., Martino, P.A., Grieco, V., Pisu, M.C., Banco B., Rota A., Veronesi, M.C., 2014. A survey on bacterial involvement in neonatal mortality in dogs. Vet. Ital. 50, 293-299. https://doi.org/10.12834/VetIt.45.2244.2

Milani, C., Corrò, M., Drigo, M., Rota, A., 2012. Antimicrobial resistance in bacteria from breeding dogs housed in kennels with differing neonatal mortality and use of antibiotics. Theriogenology 78, 1321–1328. https://doi.org/https://doi.org/10.1016/j.theriogenology.2012.05.033

Miller, L., Zawistowski, S., 2013. Shelter medicine for veterinarians and staff. Wiley-Blackwell.

Miller, L., Zawistowski, S., 2012. Housing, husbandry, and behavior of dogs in animal shelters, in: Miller, L., Zawistowski, S. (Eds.), Animal Behavior for Shelter Veterinarians and Staff. Wiley-Blackwell, Chichester, UK, p. 145. https://doi.org/10.1002/9781119421313.ch7

Miranda, C., Thompson, G., 2016. Canine parvovirus: the worldwide occurrence of antigenic variants. J. Gen. Virol. 97, 2043–2057. https://doi.org/10.1099/jgv.0.000540

Moore, G.E., Glickman, L.T., 2004. A perspective on vaccine guidelines and titer tests for dogs. J. Am. Vet. Med. Assoc. 224, 200–203.

Münnich, A., 2008. The pathological newborn in small animals: the neonate is not a small adult. Vet. Res. Commun. 32, 81–85. https://doi.org/10.1007/s11259-008-9096-0

Münnich, A., Lübke-Becker, A., 2004. Escherichia coli infections in newborn puppies—clinical and epidemiological investigations. Theriogenology.

Nagy, A., Ziadinov, I., Schweiger, A., Schnyder, M., Deplazes, P., 2011. [Hair coat contamination with zoonotic helminth eggs of farm and pet dogs and foxes]. Berl. Munch. Tierarztl. Wochenschr. 124, 503–11.

Nielen, A., Gaag, I. Van der, Knol, B., 1998. Investigation of mortality and pathological changes in a 14-month birth cohort of boxer puppies. Vet.

Nielen, A.L., van der Gaag, I., Knol, B.W., Schukken, Y.H., 1998. Investigation of mortality and pathological changes in a 14-month birth cohort of boxer puppies. Vet. Rec. 142, 602–6. https://doi.org/10.1136/VR.142.22.602

Normand, E.., Gibson, N.., Reid, S.W.., Carmichael, S., Taylor, D.., 2000. Antimicrobial-resistance trends in bacterial isolates from companion-animal community practice in the UK. Prev. Vet. Med. 46, 267–278. https://doi.org/10.1016/S0167-5877(00)00149-5

Pierantoni, L., Albertini, M., Pirrone, F., 2011. Prevalence of owner-reported behaviours in dogs separated from the litter at two different ages. Vet. Rec. 169. https://doi.org/10.1136/vr.d4967

Pomba, C., Rantala, M., Greko, C., Baptiste, K.E., Catry, B., van Duijkeren, E., Mateus, A., Moreno, M.A., Pyörälä, S., Ružauskas, M., Sanders, P., Teale, C., John Threlfall, E., Kunsagi, Z., Torren-Edo, J., Jukes, H., Törneke, K., 2016. Public health risk of antimicrobial resistance transfer from companion animals. J. Antimicrob. Chemother. 72, 957–968. https://doi.org/10.1093/jac/dkw481

Postma, M., Vanderhaeghen, W., Sarrazin, S., Maes, D., Dewulf, J., 2017. Reducing Antimicrobial Usage in Pig Production without Jeopardizing Production Parameters. Zoonoses Public Health 64, 63–74. https://doi.org/10.1111/zph.12283

Priestnall, S.L., Mitchell, J.A., Walker, C.A., Erles, K., Brownlie, J., 2014. New and Emerging Pathogens in Canine Infectious Respiratory Disease. Vet. Pathol. 51, 492–504. https://doi.org/10.1177/0300985813511130

Primm, K., 2017. Canine influenza strikes the Southeast: Another outbreak? [WWW Document]. dvm360.com. URL http://veterinarynews.dvm360.com/canine-influenza-strikes-southeast-another-outbreak

Radek, K.A., 2010. Antimicrobial anxiety: the impact of stress on antimicrobial immunity. J. Leukoc. Biol. 88, 263–77. https://doi.org/10.1189/jlb.1109740

Ronsse, V., Verstegen, J., Onclin, K., Guiot, A., Aeberle, C., Nauwynck, H., Poulet, H., 2002. Seroprevalence of Canine Herpesvirus-1 in the Belgian Dog Population in 2000. Reprod. Domest. Anim. 37, 299–304. https://doi.org/10.1046/j.1439-0531.2002.00363.x

Root Kustritz, M. V, 2011. Chapter 4 - History and Physical Examination of the Weanling and Adolescent, in: Kutzler, M.E.P.A. (Ed.), Small Animal Pediatrics. W.B. Saunders, Saint Louis, pp. 28–33. https://doi.org/http://dx.doi.org/10.1016/B978-1-4160-4889-3.00004-8

Rota, A., Milani, C., Corrò, M., Drigo, I., Börjesson, S., 2013. Misuse of Antimicrobials and Selection of Methicillin-Resistant Staphylococcus pseudintermedius Strains in Breeding Kennels: Genetic Characterization of Bacteria After a Two-year Interval. Reprod. Domest. Anim. 48, 1–6. https://doi.org/10.1111/j.1439-0531.2012.02012.x

Rota, A., Milani, C., Drigo, I., Drigo, M., Corrò, M., 2011. Isolation of methicillin-resistant Staphylococcus pseudintermedius from breeding dogs. Theriogenology 75, 115–121. https://doi.org/10.1016/j.theriogenology.2010.07.016

Scarlett, J.M., Saidla, J.E., Pollock, R. V, 1994. Source of acquisition as a risk factor for disease and death in pups. J. Am. Vet. Med. Assoc. 204, 1906–13.

Schäfer-Somi, S., Spergser, J., Breitenfellner, J., Aurich, J.E., 2003. Bacteriological status of canine milk and septicaemia in neonatal puppies--a retrospective study. J. Vet. Med. B. Infect. Dis. Vet. Public Health 50, 343–6.

Schumaker, B.A., Miller, M.M., Grosdidier, P., Cavender, J.L., Montgomery, D.L., Cornish, T.E., Farr, R.M., Driscoll, M., Maness, L.J., Gray, T., Petersen, D., Brown, W.L., Logan, J., O'Toole, D., 2012. Canine distemper outbreak in pet store puppies linked to a high-volume dog breeder. J. Vet. Diagnostic Investig. 24, 1094–1098. https://doi.org/10.1177/1040638712460531

Silva, F., Oliveira, R., Silva, T., Xavier, M., 2009. Venereal transmission of canine visceral leishmaniasis. Veterinary.

Simmons, K., Hoffman, C., 2016. Dogs on the Move: Factors Impacting Animal Shelter and Rescue Organizations' Decisions to Accept Dogs from Distant Locations. Animals 6, 11. https://doi.org/10.3390/ani6020011

Song, D., Kang, B., Lee, C., Jung, K., Ha, G., Kang, D., Park, S., Park, B., Oh, J., 2008. Transmission of avian influenza virus (H3N2) to dogs. Emerg. Infect. Dis. 14, 741–6. https://doi.org/10.3201/eid1405.071471

Speksnijder, D.C., Mevius, D.J., Bruschke, C.J.M., Wagenaar, J.A., 2015. Reduction of Veterinary Antimicrobial Use in the Netherlands. The Dutch Success Model. Zoonoses Public Health 62, 79–87. https://doi.org/10.1111/zph.12167

Stavisky, J., Pinchbeck, G., Gaskell, R.M., Dawson, S., German, A.J., Radford, A.D., 2012. Cross sectional and longitudinal surveys of canine enteric coronavirus infection in kennelled dogs: A molecular marker for biosecurity. Infect. Genet. Evol. 12, 1419–1426. https://doi.org/10.1016/j.meegid.2012.04.010

Stehr-Green, J., Murray, G., 1987. Intestinal parasites in pet store puppies in Atlanta. Am. J. Public Health 77, 345–346.

Strandberg, E., Jacobsson, J., Saetre, P., 2005. Direct genetic, maternal and litter effects on behaviour in German shepherd dogs in Sweden. Livest. Prod. Sci. 93, 33–42. https://doi.org/10.1016/j.livprodsci.2004.11.004

Sutter, N.B., Ostrander, E.A., 2004. Dog star rising: the canine genetic system. Nat. Rev. Genet. 5, 900–910.

Takahashi, L.K., Turner, J.G., Kalin, N.H., 1992. Prenatal stress alters brain catecholaminergic activity and potentiates stress-induced behavior in adult rats. Brain Res. 574, 131–137. https://doi.org/10.1016/0006-8993(92)90809-N

Terpstra, F.G., van den Blink, A.E., Bos, L.M., Boots, A.G.C., Brinkhuis, F.H.M., Gijsen, E., van Remmerden, Y., Schuitemaker, H., van 't Wout, A.B., 2007. Resistance of surface-dried virus to common disinfection procedures. J. Hosp. Infect. 66, 332–338. https://doi.org/10.1016/j.jhin.2007.05.005

Thalmann, O., Shapiro, B., Cui, P., Schuenemann, V.J., Sawyer, S.K., Greenfield, D.L., Germonpré, M.B., Sablin, M. V., López-Giráldez, F., Domingo-Roura, X., Napierala, H., Uerpmann, H.P., Loponte, D.M., Acosta, A.A., Giemsch, L., Schmitz, R.W., Worthington, B., Buikstra, J.E., Druzhkova, A., Graphodatsky, A.S., Ovodov, N.D., Wahlberg, N., Freedman, A.H., Schweizer, R.M., Koepfli, K.P., Leonard, J.A., Meyer, M., Krause, J., Pääbo, S., Green, R.E., Wayne, R.K., 2013. Complete mitochondrial genomes of ancient canids suggest a European origin of domestic dogs. Science (80-.). 342, 871–874. https://doi.org/10.1126/science.1243650

Thiry, E., Horzinek, M.C., 2007. Vaccination guidelines: a bridge between official requirements and the daily use of vaccines. Rev. Sci. Tech. 26, 511–517.

Thrusfield, M. V., Aitken, C.G.G., Muirhead, R.H., 1991. A field investigation of kennel cough: Incubation period and clinical signs. J. Small Anim. Pract. 32, 215–220. https://doi.org/10.1111/j.1748-5827.1991.tb00550.x

Tiira, K., Lohi, H., 2015. Early Life Experiences and Exercise Associate with Canine Anxieties. PLoS One 10, e0141907. https://doi.org/10.1371/journal.pone.0141907

Titulaer, M., Blackwell, E.J., Mendl, M., Casey, R.A., 2013. Cross sectional study comparing behavioural, cognitive and physiological indicators of welfare between short and long term kennelled domestic dogs. Appl. Anim. Behav. Sci. 147, 149–158. https://doi.org/10.1016/j.applanim.2013.05.001

Tuchscherer, M., Kanitz, E., Otten, W., Tuchscherer, A., 2002. Effects of prenatal stress on cellular and humoral immune responses in neonatal pigs. Vet. Immunol. Immunopathol. 86, 195–203. https://doi.org/10.1016/S0165-2427(02)00035-1

Umhang, G., Comte, S., Raton, V., Hormaz, V., Boucher, J.M., Favier, S., Combes, B., Boue, F., 2014. Echinococcus multilocularis infections in dogs from urban and peri-urban areas in France. Parasitol. Res. 113, 2219–2222. https://doi.org/10.1007/s00436-014-3875-z

Villarroel, A., Dargatz, D.A., Lane, V.M., McCluskey, B.J., Salman, M.D., 2007. Suggested outline of potential critical control points for biosecurity and biocontainment on large dairy farms. J. Am. Vet. Med. Assoc. 230, 808–819. https://doi.org/10.2460/javma.230.6.808

Welborn, L. V, DeVries, J.G., Ford, R., Franklin, R.T., Hurley, K.F., McClure, K.D., Paul, M.A., Schultz, R.D., 2011. 2011 American Animal Hospital Association canine vaccination guidelines. J. Am. Anim. Hosp. Assoc. 47, 1–42.

Wilsson, E., 1984. The social interaction between mother and offspring during weaning in German shepherd dogs: individual differences between mothers 1. Wilsson E. 1984; The social interaction between mother and offspring during weaning in German shepherd dogs: individual d. Appl. Anim. Behav. Sci. 13, 101–112. https://doi.org/https://doi.org/10.1016/0168-1591(84)90056-X

Wilsson, E., Sundgren, P.-E., 1998. Effects of weight, litter size and parity of mother on the behaviour of the puppy and the adult dog. Appl. Anim. Behav. Sci. 56, 245–254. https://doi.org/https://doi.org/10.1016/S0168-1591(97)00094-4

Wright, J.C., 1983. The effects of differential rearing on exploratory behavior in puppies. Appl. Anim. Ethol. 10, 27–34. https://doi.org/10.1016/0304-3762(83)90109-8

Zicola, A., Jolly, S., Mathijs, E., Ziant, D., Decaro, N., Mari, V., Thiry, E., 2012. Fatal outbreaks in dogs associated with pantropic canine coronavirus in France and Belgium. J. Small Anim. Pract. 53, 297–300. https://doi.org/10.1111/j.1748-5827.2011.01178.x

CHAPTER 5:
BEHAVIOURAL OBSERVATIONS OF PUPPIES IN DOG BREEDING FACILITIES

This chapter has been adapted from:

Behaviour is in the eye of the beholder: does breeder type influence puppy behaviour?

AUTHORS

Pierre-Alexandre Dendoncker[1,2,3], Tiny De Keuster[1], Flavia Midori[1], Katrien Verschueren[4], Jeroen Dewulf[3], Claire Diederich[2], Christel P.H. Moons[1],

1 Laboratory for Ethology, Department Nutrition, Genetics and Ethology, Faculty of Veterinary Medicine, Ghent University, Heidestraat 19, 9820 Merelbeke

2 Integrated Veterinary Research Unit (IVRU), Department of Veterinary Medicine, Faculty of Sciences, University of Namur, Rue de Bruxelles 61, 5000 Namur

3 Veterinary Epidemiology Unit, Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke

4 Living Statistics, Kunstenaarstraat 35, 9040 Sint-Amandsberg

Manuscript in preparation

1. ABSTRACT

This study examined differences in spontaneous behaviour and behavioural responses in puppies at different categories of breeders. 107 puppies from 23 litters were observed in their pens one week before homing, at the mean age of 9.4 weeks (SD 2.9). Behaviour was scored in three contexts: for 5 minutes in response to a novel object, for 10 minutes without any disturbance (spontaneous behaviour) and for 1 minute following an approach by a stranger. The breeder categories were a re-classification of the categories originally defined in Belgian legislation: occasional breeder (<10 dams on site; n=4), occupational breeder (10 to 50 dams on site; n=3), commercial breeder (>50 dams on site; n=3), and dog merchant (≤50 dams but selling >350 puppies/year; n=3).

Puppies from commercial breeders showed more exploration activity compared to puppies from breeders of other categories in all contexts. These puppies also spent proportionally more time investigating the novel object compared to puppies from merchants and had more interactions with littermates during the novel object disturbance. No differences in the frequency of behaviours indicative of stress signals in any of the contexts could be identified between breeders.

Our study provides an indication that puppies from the participating commercial breeders (but not dog merchants) could have a more proactive coping style compared to those from other breeder types.

2. INTRODUCTION

'A man's best friend' is a phrase that has been used for centuries to describe the popularity and versatility of dogs and the potential relationship between dog and owner. Originally, dogs fulfilled various tasks such as herding, guarding or hunting (Turcsán et al., 2011). In Victorian era, a shift occurred towards custom-breeding for phenotypes (vonHoldt et al., 2010), eventually leading to a breed standard. The current standards refer to purpose-specific physical traits, movement, and temperament (AKC, 2019). Dogs of modern times, however, are mainly kept for companionship and represent to their owners a member of the family, a friend to play or share activities with, a source of security or social support, as well as an object of care (Dotson and Hyatt, 2008; O'Connor et al., 2016). In western countries, at least 20% of all households keep at least one dog (AVMA, 2012; HAS Kennistransfer & Bedrijfsopleidingen, 2015; STATBEL, 2010). The dog owners' expectation of their dog's behaviour varies according to their lifestyle or previous experience (O'Connor et al., 2016). Unmet expectations such as inappropriate behaviour are the main cause of shelter relinquishment (Collisson, 2015; Diesel et al., 2010; Weng et al., 2006). Therefore, breeding practices must also be directed towards providing an emotionally balanced and behaviourally adaptive pet dog (King et al., 2012).

Behavioural development is affected by pre- and postnatal influences. In all mammals, it starts as early as mate choice (Lindberg et al., 2005), where the genome (Lindblad-Toh et al., 2005) draws the outline for an individual's behaviour (reviewed by van Rooy et al., 2014). Stressful experiences by the dam may transfer to the developing puppies, thereby curtailing offspring's maturation (Austin et al., 2005; Czerwinski et al., 2016). High cortisol-levels in the dam can alter the expression of the genome of the foetus, increasing the hypothalamic–pituitary–adrenal (HPA) axis activity (Zhang and Meaney, 2010). These epigenetic effects result in a disruption of normal behaviour when challenging situations are encountered later in life (Braastad, 1998). Repeated exposure of the dam to unpredictable stressful situations may also alter the expression of maternal behaviour after birth (Bosch and Neumann, 2012; Neumann et al., 2005), impacting growth and stress-response of the puppies. Good maternal care has immediate impact on endocrine function of offspring, as it stimulates growth and dampens HPA axis activity (reviewed by Meaney, 2001).

Psycho-motor and cognitive capacities evolve gradually from foetus to adulthood and are under constant influence of the environment (Gómez, 2005; Piaget, 1977). Historically, canine behavioural ontogeny has been characterised by different stages, of which the neonatal (0-2 weeks) and sensitive period (3-14 weeks) occur during the time a puppy is with its mother, littermates, and in an environment shaped by the breeder (Overall, 2013; Scott and Fuller, 1965).

The environment in which puppies are raised today varies greatly. The popularity of dogs created business opportunities for dog breeders, with an increase in scale and more international commercial trade as a result. Consequently, breeding facilities range from household environments that are enriched but vary according to circumstances, to industrialised settings within a less enriched (Dendoncker et al., 2019a) yet more controlled environment (Dendoncker et al., 2018). A controlled environment encompasses an application of biosecurity measures. However, the key principle of biosecurity is limiting social contact and complex environments (Dewulf and Van Immerseel, 2018). The execution of early socialisation and environmental learning tends to conflict with this principle (Stepita et al., 2013).

To which extent the variation in breeder environment influences the behavioural development of puppies is currently poorly documented. There is growing evidence that intensive dog breeding may be detrimental for dog breeding. As reviewed by McMillan (2017), much of this evidence is self-reported by owners (including the origin of the dog) and concerns adult dogs. As such, existing studies are faced with a number of difficulties. First, although gathering owner-declared behavioural information is broadly used in research (Diederich and Giffroy, 2006) and is useful to determine the perception of the owner and to evaluate risk-factors for an altered human-dog bond (Collisson, 2015; Duffy et al., 2014; Patronek et al., 1996; Salman et al., 2010), there are a few caveats to the method. Dog owners are mostly unaware of their puppy´s breeder category unless there is a reason to investigate the source. Classifying dog breeders by owner-declaration only will result in selection bias. Next, self-reporting is subject to response category cut-point shift (Lindeboom and van Doorslaer, 2004), implying that relation of owner-reported traits to an objective behavioural assessment may differ by owner-dependent factors (Dowd and

Zajacova, 2010). How owners perceive the behaviour of their dog is influenced by gender and age of the owner (Casey et al., 2014; Jacobs et al., 2017; Marinelli et al., 2007), by previous dog experience (Svartberg, 2002), the duration of ownership (Kubinyi et al., 2009), by socioeconomic status (Patronek et al., 1996), and the quality of dog-owner bond (reviewed by Payne et al., 2015). Second, gathering information about adult dogs to compare different breeders will introduce confounders because socialisation and environmental learning continue to affect behavioural development after homing. In this respect, it is known that people who buy a puppy from registered small-scale breeders (i.e., kennel club breeders) invest significantly more time and money when acquiring a puppy compared to buyers of other breeder types (Dendoncker et al., 2019a). This trend seems to continue in further investments, such as more awareness of problematic behaviour, more training courses and daily walks (Pirrone et al., 2016), but also more previous experience, and use of better training techniques were observed (Blackwell et al., 2008). Therefore, by assessing behaviour at adult age, we not only measure the differences attributable to the breeder but also differences related to ownership and the associated context in which the puppy was raised.

A more constructive approach appears the assessment of differences in behaviour through systematic observations (Tami and Gallagher, 2009). Similar to children (Ainsworth, 1969; Fox and Stelzner, 1966), the locomotion, level of activity and exploratory behaviour of dogs, referred to as exploration activity (Fox and Spencer, 1969), and their response towards nonsocial and intraspecific (Previde et al., 2009) or interspecific social stimuli (Rooney et al., 2000) have been described in working dogs as proxies for the future ability to function and adapt in human society (Asher et al., 2013; Koda, 2001; McGarrity et al., 2016). Additionally, a longitudinal study revealed that exploration activity in pet dogs was consistent between the puppy at six weeks in the breeding facility and the adult test at two years with their owner (Riemer et al., 2014).

Research in other species has demonstrated that parallels exist between activity and the strategy applied by an animal to cope with a stressor (Koolhaas et al., 2010). Two coping styles, characterised by consistent behavioural and neuroendocrine characteristics, have been described: proactive and reactive. These coping styles are influenced by genetic as

well as non-genetic factors (Koolhaas et al., 1999). In humans, evidence exist that the exploratory behaviour is determinant of future behaviour: a higher neonate activity is correlated with openness to new experiences in children (Korner, 1971). Likewise, more exploration activity was observed in proactive great tits (Verbeek et al., 1994). Similar effects are expected in dogs. In female puppies for instance, lower activity was associated with shyness (Beaudet et al., 1994).

This study aimed to record behavioural differences between puppies raised at the different breeder categories to identify variability in spontaneous behaviour and behavioural responses as an indicator for the behavioural tendency and coping style. Additionally, this study permitted a comparison between the different categories of breeders without owner-related confounders.

3. MATERIALS AND METHODS

This study was subjected to EU regulation (Directive 2010/63/EU) and approval was sought from the Ethical Committee of the Faculty of Veterinary Medicine at Ghent University (EC 2017/26).

3.1. Study design and subjects

This study is part of a multidisciplinary longitudinal study, conducted in Belgium, from March to June 2018. The first author visited 17 breeders approximately one week before expected homing of the puppies. Visits were planned at the convenience of the breeder, while aiming for approximately one hour after the daily cleaning, feeding and care routine. Breeders were selected by random stratified sampling. Strata were four previously identified categories: occasional breeders (less than 10 dams on site), occupational breeders (between 10 and 50 dams on site), commercial breeders (over 50 dams on site), and dog merchants (50 or less dams on site and over 350 puppies sold yearly. In this manuscript, we also refer to occasional and occupational breeders as small-scale breeders and to professional breeders and dog merchants as large-scale breeders. The sampling frame consisted of 102 previously described breeding facilities (Dendoncker et al., 2018, 2019a). Thirteen breeders

that had a litter available agreed to participate. When more than one litter was present at a breeding facility, randomisation was carried out to decide which litters to observe.

3.2. Experimental setup and data collection

Puppies reported by the breeder to be separated from the dam for at least 5 days, were observed in their home pen in three different contexts. First, the puppies were exposed to a novel object, which was a tripod with remote-controlled camera (Hero 4, GoPro, GoPro, Inc., San Mateo, California USA) that was installed in the pen for video recording. This part of the observation lasted 5 minutes (novel object disturbance), because dogs show an increase in activity the first minutes after installation of a camera (Lefebvre et al., 2010). Next, the puppies were observed for another 10 minutes (spontaneous behaviour recording). During the novel object test and the recording of spontaneous behaviour, no humans were present near the pen. Finally, the first author approached and entered the pen, and stood passively in the corner for 1 minute (stranger disturbance). The novel object was cleaned between experiments and only manipulated with gloves to minimise olfactory contamination.

Data collection was performed using focal animal sampling with continuous recording. A modified ethogram (table 5.1), was compiled (Titulaer et al., 2013; Wright, 1983) that consisted of events (behaviours of short duration, such as yawning or blinking the eyes) and states (behaviours of longer duration which can last minutes, such as sleeping or walking around). Behaviours were coded with The Observer XT10 (Noldus, The Netherlands) by one trained operator (Intra-observer reliability >0.9; inter-observer reliability with first author: 0.8), who was blinded to the breeder categories that the puppies belonged to.

Table 5.1: Ethogram (modified from Titulaer et al., 2013; Wright, 1983)	
Behaviour	**Duration**
Walking towards (environment, littermate, novel object, stranger)	State
Walking away (environment, littermate, novel object, stranger)	State
Standing still	State
Standing on hind legs against (environment, littermate, novel object, stranger)	State
Sitting	State
Sleeping	State
Lying down	State
Lying on its back towards (littermate, novel object, stranger)	State
Looking at (environment, littermate, novel object, stranger)	Event
Sniffing at (environment, littermate, novel object, stranger)	Event
Licking at (environment, littermate, novel object, stranger)	Event
Giving paw to (littermate, novel object, stranger)	Event
Turning head away from (littermate, novel object, stranger)	Event
Turning body away from (littermate, novel object, stranger)	Event
Low posture/ ears flat towards	Event
Tongue flicking/ Lip licking (littermate, novel object, stranger)	Event
Showing teeth (littermate, novel object, stranger)	Event
Eating	Event
Drinking	Event
Yawning	Event
Blinking	Event
Scratching/ grooming	Event
Aggression (biting at, snapping at) (littermate, stranger)	State
Play (littermate, stranger)	State

3.3. Statistical analysis

Data were analysed at puppy level. Events were expressed as frequency (occurrence per minute) while for states, the relative duration per behaviour was calculated with a correction for the time a puppy was out of sight. Raw data were processed and descriptive statistics carried out using MS Excel (Excel 2016, Microsoft). Hierarchical linear models were fitted by means of PROC MIXED analysis in SAS 9.4 (SAS Institute) to compare variables between breeder categories, while taking into account dependence between puppies in the same

litter. We also examined the effect of puppy age. As a result, breeder category and age were included in the model as fixed effects. When age was not significant, it was removed from the model. Litter ID nested within breeder ID was included as random effect. Post-hoc pairwise comparisons of least squares means between breeder categories were performed, using Tukey-Kramer to correct for multiple comparisons.

When model residuals were not normally distributed, log10 transformation was applied and a new analysis performed. This was the case for: *interaction with littermates* (in the contexts of novel object disturbance and spontaneous behaviour), *interaction with novel object* (in the context of novel object disturbance) and *number of social conflict signals* (in the contexts of novel object disturbance, spontaneous behaviour and stranger disturbance). For all statistical tests, a confidential interval of 0.95 was retained and significance was set at a corrected P-value of 0.05.

Some behaviours occurred infrequently and resulted in variables with very low variation. The variables that were highly related to one another conceptually were combined to create new meaningful composite variables thereby permitting analysis. ”walking towards” and “standing on hind legs against” were grouped into “exploration activity”. “Sleeping”, “sitting”, “lying down” were grouped into ”passive”. Another new behaviour, “interactions”, was computed by pooling “looking at”, “sniffing at”, “licking at”, “giving paw to” and “playing with”. Finally, the most observed displacement signals and social stress signals (Shepherd, 2009), namely “low posture/ears flat towards”, “tongue flicking / lip licking”, and “showing teeth” were grouped into “stress signals”. Other less observed behaviours were discarded for analysis: “Walking away”, “Standing still”, “Lying on its back towards”, “Turning head away from”, “Turning body away from”, “Aggression (biting at, snapping at…)”, “Eats”, “Drinks”, “Yawns”, “Blinks” and “Scratching/grooming”.

4. RESULTS

We recorded the behaviour of 107 puppies from 13 different breeders. The distributions relative to the breeder category of the number of puppies, the number of litters and of puppy age are listed in table 5.2. Puppies from merchants were significantly older compared to puppies from other breeder categories ($F_{3,103}$=17.1; P<0.01).

Table 5.2: Mean age and distribution of puppies per breeder category

	Small-scale breeders		Large-scale breeders	
	Occasional breeder (n=4)	Occupational breeder (n=3)	Commercial breeder (n=3)	Merchant (n=3)
Number of puppies observed	19	31	24	33
Number of litters observed	4	6	6	7
Mean age in weeks (SD)	9.2[a] (2.5)	8.1[a] (1.8)	8[a] (0.0)	11.9[b] (3.5)

[a-b] Different indexes indicate a significant difference (P≤0.05) between breeder categories for the LSMEANS.

The mean relative durations for exploration activity and passive behaviour per breeder category are listed in table 5.3. There was a significant effect of breeder category for exploration activity in the contexts of the novel object disturbance ($F_{3,84}$=6.4; P<0.01) and of the spontaneous behaviour recording ($F_{3,84}$=5.3; P<0.01). There was also a trend in the stranger disturbance context ($F_{3,84}$=2.5; P=0.06). Post-hoc analysis revealed that puppies from commercial breeders showed more exploration activity during the novel object disturbance compared to puppies from occasional breeders (t_{84}=3.6; P<0.01), occupational breeders (t_{84}=3.3; P<0.01), and merchants (t_{84}=4.1; P<0.01). These puppies also showed more exploration activity in the spontaneous behaviour contexts compared to puppies from occasional breeders (t_{84}=3.0; P=0.02), occupational breeders (t_{84}=3.0; P=0.02), and merchants (t_{84}=3.7; P<0.01). Finally, puppies from professional breeders performed more exploration activity than those of dog merchants in the stranger disturbance context (t_{84}=2.7; P < 0.05). Age was significant in the context of stranger disturbance, and was included in the statistical model as fixed effect. The relative duration of exploration activity (i.e., mean response) increased with 5 units for every increase in age by 1 week during the

stranger disturbance ($F_{1,84}$=6.6; P=0.01). No significant differences in passive behaviour were found between breeder categories.

Table 5.3: Mean relative duration (%) of exploration activity and passive behaviour by puppies (n = 107) in three contexts, per breeder category.

	Small-scale breeders		Large-scale breeders	
	Occasional breeder (n=4)	Occupational breeder (n=3)	Commercial breeder (n=3)	Merchant (n=3)
Mean relative duration in % (SD) of exploration activity				
Novel object	4.7[a] (10.0)	9.6[a] (13.5)	39.2[b] (25.2)	4.0[a] (8.2)
Spontaneous	5.2[a] (7.8)	7.8[a] (7.3)	14.0[a] (19.1)	4.0[a] (7.5)
Stranger*	7.3[a,b] (22.1)	19.5[a,b] (22.4)	23.5[a] (40.8)	15.4[b] (22.7)
Mean relative duration % (SD) of passive behaviour				
Novel object	69.8[a] (36.5)	80.9[a] (25.8)	44.5[a] (31.7)	79.7[a] (36.4)
Spontaneous	89.1[a] (32.7)	75.8[a] (19.4)	72.7[a] (33.8)	77.2[a] (38.0)
Stranger	63.7[a] (34.6)	67.1[a] (46.0)	47.4[a] (52.3)	57.3[a] (44.9)

* Mean response increased with 5% for every increase in age by 1 week ($F_{1,84}$=6.6; P=0.01).

[a-b] Different indexes indicate a significant difference (P≤0.05) between breeder categories for the LSMEANS.

The mean relative durations of interactions with environment and littermates in the contexts of novel object disturbance and spontaneous behaviour and per breeder category are listed in table 5.4. There was a significant effect of breeder category for interaction with littermates in the context of the novel object disturbance ($F_{3,84}$=3.6; P=0.02). There was also a trend in the stranger disturbance context ($F_{3,84}$=2.2; P=0.09). Post-hoc analysis revealed that puppies from commercial breeders performed more interaction with their littermates compared to puppies from merchants (t_{84}=3.3; P<0.01) during the context of novel object disturbance and tended to interact much more (t_{84}=2.5; P=0.07) during the context of spontaneous behaviour.

Table 5.4: Mean relative duration (%) of interactive behaviours by puppies in contexts of novel object and spontaneous behaviour (n=107), per breeder category

	Small-scale breeders		Large-scale breeders	
	Occasional breeder (n=4)	Occupational breeder (n=3)	Commercial breeder (n=3)	Merchant (n=3)
Mean relative duration (%) of interaction with the environment				
Novel object	23.5^{a} (17.2)	27.2^{a} (37.5)	45.9^{a} (19.5)	15.0^{a} (26.1)
Spontaneous	17.8^{a} (29.3)	20.5^{a} (24.2)	18,0^{a} (16.7)	11.4^{a} (15.3)
Mean relative duration (%) of interaction with littermates				
Novel object	4.0^{a,b} (8.5)	4.8^{a,b} (14.8)	4.8^{a} (5.8)	1.2^{b} (3.3)
Spontaneous	3.0^{a} (4.8)	4.3^{a} (6.3)	6.2^{a} (10.0)	2.6^{a} (6.2)

$^{a-b}$ Different indexes indicate a significant difference (P≤0.05) between breeder categories for the LSMEANS.

The mean relative durations of interactive behaviours with novel object and stranger in their respective contexts per breeder category are listed in table 5.5. There was a significant effect of breeder category for interaction with novel object in the context of the novel object disturbance ($F_{3,84}$=4.1; P<0.01). Post-hoc analysis revealed that puppies from commercial breeders performed more interactions with the novel object during the context of novel object disturbance slightly more compared to puppies from occupational breeders (t_{84}=3.4; P<0.01) and compared to puppies from merchants (t_{84}=2.7; P=0.05)

Table 5.5: Mean relative duration (%) of interaction by puppies (n=107) with either the novel object or the stranger, per breeder category

	Small-scale breeders		Large-scale breeders	
	Occasional breeder (n=4)	Occupational breeder (n=3)	Commercial breeder (n=3)	Merchant (n=3)
Novel object	5.7^{a,b} (9.2)	0.7^{a} (1.8)	17.9^{b} (16.8)	4.8^{a} (11.9)
Stranger	25.3^{a} (39.9)	48.6^{a} (41.4)	34.5^{a} (49.8)	26.7^{a} (33.9)

$^{a-b}$ Different indexes indicate a significant difference (P≤0.05) between breeder categories for the LSMEANS.

The mean frequency of stress signals expressed by puppies of each breeder category are listed in table 5.6. Overall, few stress signals were recorded, and no significant effect of breeder category was observed in the contexts of novel object disturbance ($F_{3,84}$=0.2;

P=0.9), spontaneous behaviour ($F_{3,84}$=0.4; P=0.8), or stranger disturbance ($F_{3,84}$=1.0; P=0.4). Anecdotally, behavioural inhibition (freezing) meaning that all animals of one litter showed no behavioural response across all three contexts was seen in one litter, at a dog merchant's facility.

Table 5.6: Mean (SD) frequency (number per minute) of stress signals expressed by puppies (n=107) in each context and according to breeder category

	Small-scale breeders		Large-scale breeders	
	Occasional breeder (n=4)	Occupational breeder (n=3)	Commercial breeder (n=3)	Merchant (n=3)
Novel object	0.6[a] (0.9)	0.3[a] (0.6)	0.3[a] (0.4)	0.6[a] (2.6)
Spontaneous	0.4[a] (0.6)	0.1[a] (0.4)	0.1[a] (0.2)	0.5[a] (1.3)
Stranger	0.1[a] (0.2)	0.1[a] (0.3)	0.2[a] (0.5)	1.5[a] (2.9)

[a] Different indexes indicate a significant difference (P≤0.05) between breeder categories for the LSMEANS.

5. DISCUSSION

To the authors' knowledge, this is the first study that compares the behaviour of puppies from randomly selected breeders of different categories by conducting systematic observations before homing.

In this research, puppies from commercial breeders demonstrated significantly more exploration activity compared to puppies from all other breeders, and these results confirm the findings of authors describing more explorative locomotion behaviour in puppies raised by commercial breeders compared to puppies raised in a household (Gazzano et al., 2008). In our previous research, where puppies were observed during the first consultation in a veterinary practice, we also found higher activity levels for puppies from commercial breeders compared to the ones from occupational breeders (Dendoncker et al., 2019b).

In previous chapters, differences in environment have been described. Large-scale breeders provided a less enriched and stimulating environment compared to small-scale breeders. It could be hypothesised that the lack of stimulation in puppyhood creates a sibling rivalry. This competition between siblings for diverse resources, observed during food-deprivation

in several species (Carere et al., 2005) or through birth-order in humans (Damian and Roberts, 2015), results in a more proactive coping style.

Parallels exist between activity level and coping style on one hand and aggressiveness/fearfulness and a proactive/reactive behaviour style on the other hand (Koolhaas et al., 2010). Subsequently, it was also suggested that reactive dogs could demonstrate more fearful behaviour while, on the contrary, proactive dogs may present increased aggressiveness (Horváth et al., 2007; Riemer et al., 2014). In dogs however, the effect of the coping style on sociability and functionality has been less investigated and the few results tend to be contradictory. In working dogs for instance, coping style is a determinant or future abilities and training success (Svartberg, 2002). Furthermore, it has been reported that reactive dogs have a more impaired welfare in stressful conditions (Corsetti et al., 2018). Contrary to humans however, boldness and higher exploration of novel objects in puppies was not necessarily associated with higher sociability (Riemer et al., 2013). This apparent contradiction can be explained because the relationship between locomotion, activity level, and exploratory behaviour on one hand and fearfulness and stress on the other is not straightforward (Goddard and Beilharz, 1984). Therefore, differences in explorative locomotion observed in this study could also be explained by underlying motivations, such as hunger, reproductive drive, exploration, and fear or anxiety. Moderate fearful stimuli and stressful situations will augment a dog's exploratory behaviour and activity level. Past a certain individual threshold (Koolhaas et al., 2010), the activity level of the dog will decrease, eventually leading to freezing (Russell, 1973). More research is needed to determine how higher exploration activity in puppies and associated coping style translates into its future ability to adapt in human society as a pet dog.

In this study, puppies from merchants showed less interactions during the novel object disturbance compared to puppies from commercial breeders. They investigated the novel object for a shorter time and interacted less with littermates during this period. Puppies from merchants also showed behavioural inhibition. A possible explanation could be that they were recently acquired from other breeders (Dendoncker et al., 2019a, 2018), and were, therefore, not tested in the pen they were raised in, contrary to puppies from all other breeder categories.

Puppies from merchants were older compared to puppies from other breeder categories, which was to be expected due to half of the merchants having declared to acquire dogs from abroad (Dendoncker et al., 2018) and the regulations surrounding intracommunity transport of dogs (EU 577/2013). Despite the fact that for all puppies higher age was associated with increased exploration activity in the context of stranger disturbance, puppies from merchants were still less active than puppies from commercial breeders in this specific context. We found no other significant differences imputable to age. A positive correlation between exploration activity and age was observed in test-retest studies, although increase could be an experiential effect (Fox and Spencer, 1969; Riemer et al., 2014). Nevertheless, an age-bias may be present when comparing puppies from merchants to puppies form other breeder categories. A higher homing age may also be a risk factor for future behavioural impairments such as avoidance or some types of aggression (Jokinen et al., 2017).

In this study, no significant differences in passive behaviour were found between breeder categories. Passive behaviour or inactivity can be a sign of relaxation, but it could also be due to poor housing conditions that either lack suitable stimuli to elicit behaviour, or that lack adequate space to perform behaviour. Research in other species has shown that prenatal stress in the mother can also result in more passive offspring (Weinstock, 1997). In humans, passiveness is an indicator of negative emotion and similar principles may apply to dogs (Beerda et al., 1997). The assessment of passive behaviour has been suggested to be a useful indicator of impaired welfare in laboratory settings (Hubrecht et al., 1992; Spangenberg et al., 2006). In our puppies, we recorded a low number of stress signals and no significant differences were found between breeder types. One possible interpretation is that the environment was not experienced as being very stressful by the observed puppies.

Concerning the methodology, our study took a different path than other recently published studies that impute large-scale dog breeding as a risk factor for later behavioural impairments (Gray et al., 2016; McMillan et al., 2013). Instead of using owner-directed questionnaires at adult age, we performed systematic observations of the puppies before homing. The observation scheme was based on two previously published ethograms (Titulaer et al., 2013; Wright, 1983). These ethograms had been used in studies with a similar

kennel setup or with puppies at a similar age and provided us with a broad overview of performed behaviors. Systematic observation was preferred to owner-directed questionnaire because the external validity of the latter is questionable: first, a combination of non-probability sampling techniques is common practice, such as self-selection, snowball, respondent-driven and target sampling. The choice of convenience sampling will inherently induce a bias (Cochran, 1977). This is especially the case when the various breeder categories are submitted to different sampling techniques: for instance, target sampling of rehomed dogs that originate from large-scale breeders versus self-selection sampling of control dogs (McMillan et al., 2011; Wauthier et al., 2018). Second, studies based on convenience sampling after homing generally rely on owner-declared origin (Bennett and Rohlf, 2007; Flannigan and Dodman, 2001; Pierantoni et al., 2011). However, anecdotal reports from veterinarians in the field, posts from dog owners on social media or enthusiast groups, and interviews in newspapers show that most dog owners are generally unaware of the origin of their puppy (Marder and Duxbury, 2008), and more specifically of the scale at which the breeder operates, until they have a motivation to investigate it. The large amount of owners that choose *unsure* (Wauthier et al., 2018) or *other* (Martínez et al., 2011; Salman et al., 2010) instead of *breeder* or *pet store* as origin confirms this. Additionally, some large-scale breeders were found to use misleading communication namely: *raised in a household*, *raised by a small-scale familial* (personal observation), to counter the negative connotation of their activities and attract more potential buyers. It stands to reason that consequently, some owners will be misinformed on the origin of their puppy, which might lead to questionable results of owner-based questionnaires with self-reported acquisition source.

Some limitations did apply to this study. First and foremost, participation was based on willingness of the breeder to participate. Because this study was a revisit, the randomly selected breeders were eager to contribute, which resulted in an exceptional participation rate, but also in a small sample size. As such, representativeness for the total population can’t be guaranteed, but the stratified random sampling limits selection bias (Cochran, 1977). Second, visits were announced and therefore, animals presenting problematic behaviour may have been removed by some breeders. Third, direct observations occurred for a limited time only (16 minutes per litter) and were performed in the pen where puppies

are raised. As described in our previous study (Dendoncker et al., 2019a), housing of puppies varies greatly between breeders (ranging from a pen in a living room to a glass showroom in a pet store) and the differences between observation arenas could have interfered with our observations. However, all puppies (except from merchants) were acquainted with their environment. Fourth, the age of interest in this study also covered the onset of fear-avoidance behaviour (Morrow et al., 2015). This critical stage during socialisation is generally accepted to occur for about one week between the ages of 8-12 weeks (Coppinger and Coppinger, 2001). This could have influenced the level of fear in puppies observed during this one time point. Last, stress signals were coded as event, which did not allow to measure duration. Behaviours that generally are longer in duration, such as freezing, could therefore not be analysed quantitatively. Stress may, therefore, have been underestimated in our study. It would be advisable in future research to perform longer observation of stress and also to include a measurement of stress related behaviour (e.g., by summing al stress-related events, or by coding the duration of the low posture, ears flat and freezing) as a duration.

Testing of juveniles has been the subject of controversy (Wilsson and Sundgren, 1998), and most studies were not able to demonstrate a personality consistency in dogs. Recently however, a meta-analysis of these published studies did demonstrate a certain consistency of personality throughout the dog's life (Fratkin et al., 2013). Early observations of puppies, therefore, may provide some valuable information concerning behavioural tendencies and coping style. Nevertheless, it remains advisable to interpret results of early testing of juveniles with caution. First, personality consistency is lower in puppies and increases with age. Second, while the importance of a sensitive period for socialisation in young puppies is often stressed, this does not imply that environmental influences that occur at other developmental stages do not have effects as well (Riemer et al., 2014). Therefore, it is beneficial to all that both the breeder, regardless of its category, and the owner should raise and socialise the puppy in a controlled environment, led by current scientific consensus, in order to maximise positive experiences and limit future behavioural impairments.

6. CONCLUSION

We compared different types of dog breeders by observing the behaviour of puppies before homing at their breeding facility. In our study, dogs from commercial breeders seemed to be more explorative. Our results suggest that commercial breeders produce more proactive puppies compared to the puppies from other breeder categories. More research is needed to see how this translates into the ability to discover the environment and socialise, or could predispose to future behavioural impairments. Meanwhile, it may be more judicious to guide all breeders towards better practices while improving communication with future dog owners on good practices of how to raise their puppy, than to focus on a breeder type as being the source of future behavioural impairment.

7. REFERENCES

Ainsworth, M.D.S., 1969. Object Relations, Dependency, and Attachment: A Theoretical Review of the Infant-Mother Relationship. Child Dev. 40, 969. https://doi.org/10.2307/1127008

AKC, 2019. Dog Breeds - Types Of Dogs - American Kennel Club [WWW Document]. URL https://www.akc.org/dog-breeds/ (accessed 3.19.19).

Asher, L., Blythe, S., Roberts, R., Toothill, L., Craigon, P.J., Evans, K.M., Green, M.J., England, G.C.W., 2013. A standardized behavior test for potential guide dog puppies: Methods and association with subsequent success in guide dog training. J. Vet. Behav. Clin. Appl. Res. 8, 431–438. https://doi.org/10.1016/j.jveb.2013.08.004

Austin, M.P., Leader, L.R., Reilly, N., 2005. Prenatal stress, the hypothalamic-pituitary-adrenal axis, and fetal and infant neurobehaviour. Early Hum. Dev. 81, 917–926. https://doi.org/10.1016/j.earlhumdev.2005.07.005

AVMA, 2012. U.S. pet ownership and demographics sourcebook. American Veterinary Medical Association, Schaumburg, IL.

Beaudet, R., Chalifoux, A., Science, A.D.-A.A.B., 1994, undefined, Dallaire, A., 1994. Predictive value of activity level and behavioral evaluation on future dominance in puppies. Appl. Anim. Behav. Sci. 40, 273–284. https://doi.org/10.1016/0168-1591(94)90068-X

Beerda, B., Schilder, M.B.H., van Hooff, J.A.R.A.M., de Vries, H.W., 1997. Manifestations of chronic and acute stress in dogs. Appl. Anim. Behav. Sci. 52, 307–319. https://doi.org/10.1016/S0168-1591(96)01131-8

Bennett, P.C., Rohlf, V.I., 2007. Owner-companion dog interactions: Relationships between demographic variables, potentially problematic behaviours, training engagement and shared activities. Appl. Anim. Behav. Sci. 102, 65–84. https://doi.org/10.1016/J.APPLANIM.2006.03.009

Blackwell, E.J., Twells, C., Seawright, A., Casey, R.A., 2008. The relationship between training methods and the occurrence of behavior problems, as reported by owners, in a population of domestic dogs. J. Vet. Behav. Clin. Appl. Res. 3, 207–217. https://doi.org/10.1016/j.jveb.2007.10.008

Bosch, O.J., Neumann, I.D., 2012. Both oxytocin and vasopressin are mediators of maternal care and aggression in rodents: From central release to sites of action. Horm. Behav. 61, 293–303. https://doi.org/10.1016/J.YHBEH.2011.11.002

Braastad, B.O., 1998. Effects of prenatal stress on behaviour of offspring of laboratory and farmed mammals. Appl. Anim. Behav. Sci. 61, 159–180. https://doi.org/10.1016/S0168-1591(98)00188-9

Carere, C., Drent, P.J., Koolhaas, J.M., Groothuis, T.G.G., 2005. Epigenetic effects on personality traits: early food provisioning and sibling competition. Behaviour 142, 1329–1355. https://dx.doi.org/10.1163/156853905774539328

Casey, R.A., Loftus, B., Bolster, C., Richards, G.J., Blackwell, E.J., 2014. Human directed aggression in domestic dogs (Canis familiaris): Occurrence in different contexts and risk factors. Appl. Anim. Behav. Sci. 152, 52–63. https://doi.org/10.1016/j.applanim.2013.12.003

Cochran, W.G., William G., 1977. Sampling techniques. Wiley.

Collisson, B., 2015. Farewell to Fido : Pet Owners ' Commitment and Relinquishment of Companion Animals. Am. J. Appl. Psychol. 3, 27–33. https://doi.org/10.12691/ajap-3-2-1

Coppinger, R., Coppinger, L., 2001. Dogs :a startling new understanding of canine origin, behavior, and evolution. Scribner, New York.

Corsetti, S., Borruso, S., Di Traglia, M., Lai, O., Alfieri, L., Villavecchia, A., Cariola, G., Spaziani, A., Natoli, E., 2018. Bold personality makes domestic dogs entering a shelter less vulnerable to diseases. PLoS One 13, e0193794. https://doi.org/10.1371/journal.pone.0193794

Czerwinski, V.H., Smith, B.P., Hynd, P.I., Hazel, S.J., 2016. The influence of maternal care on stress-related behaviors in domestic dogs: What can we learn from the rodent literature? J. Vet. Behav. 14, 52–59. https://doi.org/10.1016/J.JVEB.2016.05.003

Damian, I.R., Roberts, W., 2015. The associations of birth order with personality and intelligence in a representative sample of U.S. high school students. J. Res. Pers. 58, 96-105. https://doi.org/10.1016/j.jrp.2015.05.005

Dendoncker, P.-A., De Keuster, T., Diederich, C., Dewulf, J., Moons, C., 2019a. On the origin of puppies: Breeding and selling procedures relevant for canine behavioural development. Vet. Rec. https://doi.org/http://dx.doi.org/10.1136/vr.104979

Dendoncker, P.-A., Moons, C., Sarrazin, S., Diederich, C., Thiry, E., de Keuster, T., Dewulf, J., 2018. Biosecurity and management practices in different dog breeding systems have considerable margin for improvements. Vet. Rec. 183. https://doi.org/10.1136/vr.104996

Dendoncker, P.-A., Moons, C.P.H., De Keuster, T., Lensen, C.M.M., Dewulf, J., Thiry, E., Diederich, C., 2019b. From breeding facility to the owners'home. Vet. J.

Dewulf, J., Van Immerseel, F., 2018. Biosecurity in animal production and veterinary medicine : From principles to practice., first edit. ed.

Diederich, C., Giffroy, J.-M., 2006. Behavioural testing in dogs: A review of methodology in search for standardisation. Appl. Anim. Behav. Sci. 97, 51–72. https://doi.org/http://dx.doi.org/10.1016/j.applanim.2005.11.018

Diesel, G., Brodbelt, D., Pfeiffer, D.U., 2010. Characteristics of Relinquished Dogs and Their Owners at 14 Rehoming Centers in the United Kingdom. J. Appl. Anim. Welf. Sci. 13, 15–30. https://doi.org/10.1080/10888700903369255

Döring, D., Roscher, A., Scheipl, F., Küchenhoff, H., Erhard, M.H., 2009. Fear-related behaviour of dogs in veterinary practice. Vet. J. 182, 38–43. https://doi.org/10.1016/J.TVJL.2008.05.006

Dotson, M.J., Hyatt, E.M., 2008. Understanding dog–human companionship. J. Bus. Res. 61, 457–466. https://doi.org/10.1016/j.jbusres.2007.07.019

Dowd, J.B., Zajacova, A., 2010. Does Self-Rated Health Mean the Same Thing Across Socioeconomic Groups? Evidence From Biomarker Data. Ann. Epidemiol. 20, 743–749. https://doi.org/10.1016/J.ANNEPIDEM.2010.06.007

Duffy, D.L., Kruger, K.A., Serpell, J.A., 2014. Evaluation of a behavioral assessment tool for dogs relinquished to shelters. Prev. Vet. Med. 117, 601–609. https://doi.org/http://dx.doi.org/10.1016/j.prevetmed.2014.10.003

Flannigan, G., Dodman, N.H., 2001. Risk factors and behaviors associated with separation anxiety in dogs. J. Am. Vet. Med. Assoc. 219, 460–466. https://doi.org/10.2460/javma.2001.219.460

Fox, M.W., Spencer, J.W., 1969. Exploratory behavior in the dog: Experiential or age dependent? Dev. Psychobiol. 2, 68–74. https://doi.org/10.1002/dev.420020205

Fox, M.W., Stelzner, D., 1966. Behavioural effects of differential early experience in the dog. Anim. Behav. 14, 273–281. https://doi.org/10.1016/S0003-3472(66)80083-0

Fratkin, J.L., Sinn, D.L., Patall, E.A., Gosling, S.D., 2013. Personality Consistency in Dogs: A Meta-Analysis. PLoS One 8, e54907. https://doi.org/10.1371/journal.pone.0054907

Gazzano, A., Mariti, C., Notari, L., Sighieri, C., McBride, E.A., 2008. Effects of early gentling and early environment on emotional development of puppies. Appl. Anim. Behav. Sci. 110, 294–304. https://doi.org/10.1016/j.applanim.2007.05.007

Goddard, M.E., Beilharz, R.G., 1984. The relationship of fearfulness to, and the effects of, sex, age and experience on exploration and activity in dogs. Appl. Anim. Behav. Sci. 12, 267–278. https://doi.org/10.1016/0168-1591(84)90119-9

Gómez, J.-C., 2005. Species comparative studies and cognitive development. Trends Cogn. Sci. 9, 118–125. https://doi.org/10.1016/J.TICS.2005.01.004

Gray, R., Butler, S., Douglas, C., Serpell, J.A., 2016. Puppies from “puppy farms” show more temperament and behavioural problems than if acquired from other sources, in: Recent Advances in Animal Welfare Science V: UFAW Animal Welfare Conference. Newcastle University, p. 58.

HAS Kennistransfer & Bedrijfsopleidingen, 2015. Feiten & Cijfers Gezelschapsdierensector 2015, HAS Hogeschool & Utrecht University faculty of Veterinary medicine. Den Bosch.

Horváth, Z., Igyártó, B.-Z., Magyar, A., Miklósi, Á., 2007. Three different coping styles in police dogs exposed to a short-term challenge. Horm. Behav. 52, 621–630. https://doi.org/10.1016/J.YHBEH.2007.08.001

Hubrecht, R.C., Serpell, J.A., Poole, T.B., 1992. Correlates of pen size and housing conditions on the behaviour of kennelled dogs. Appl. Anim. Behav. Sci. 34, 365–383. https://doi.org/10.1016/S0168-1591(05)80096-6

Jacobs, J.A., Pearl, D.L., Coe, J.B., Widowski, T.M., Niel, L., 2017. Ability of owners to identify resource guarding behaviour in the domestic dog. Appl. Anim. Behav. Sci. 188, 77–83. https://doi.org/10.1016/j.applanim.2016.12.012

Jokinen, O., Appleby, D., Sandbacka-Saxén, S., Appleby, T., Valros, A., 2017. Homing age influences the prevalence of aggressive and avoidance-related behaviour in adult dogs. Appl. Anim. Behav. Sci. 195, 87–92. https://doi.org/10.1016/J.APPLANIM.2017.06.003

King, T., Marston, L.C., Bennett, P.C., 2012. Breeding dogs for beauty and behaviour: Why scientists need to do more to develop valid and reliable behaviour assessments for dogs kept as companions. Appl. Anim. Behav. Sci. 137, 1–12. https://doi.org/10.1016/J.APPLANIM.2011.11.016

Koda, N., 2001. Development of play behavior between potential guide dogs for the blind and human raisers. Behav. Processes 53, 41–46. https://doi.org/10.1016/S0376-6357(00)00146-7

Koolhaas, J., Korte, S., De Boer, S., Van Der Vegt, B., Van Reenen, C., Hopster, H., De Jong, I., Ruis, M.A., Blokhuis, H., 1999. Coping styles in animals: current status in behavior and stress-physiology. Neurosci. Biobehav. Rev. 23, 925–935. https://doi.org/10.1016/S0149-7634(99)00026-3

Koolhaas, J.M., de Boer, S.F., Coppens, C.M., Buwalda, B., 2010. Neuroendocrinology of coping styles: Towards understanding the biology of individual variation. Front. Neuroendocrinol. 31, 307–321. https://doi.org/10.1016/j.yfrne.2010.04.001

Korner, A.F., 1971. Individual differences at birth: implications for early experience and later development. Am. J. Orthopsychiatry 41, 608–19.

Kubinyi, E., Turcsán, B., Miklósi, Á., 2009. Dog and owner demographic characteristics and dog personality trait associations. Behav. Processes 81, 392–401. https://doi.org/10.1016/J.BEPROC.2009.04.004

Lefebvre, D., Depiereux, E., Giffroy, J.-M., Diederich, C., 2010. Research note: Installation of videorecording equipment had minimal effect on behavioral measurements in 14 kenneled military dogs. J. Vet. Behav. 5, 2–6. https://doi.org/10.1016/J.JVEB.2009.09.044

Lindberg, J., Björnerfeldt, S., Saetre, P., Svartberg, K., Seehuus, B., Bakken, M., Vilà, C., Jazin, E., 2005. Selection for tameness has changed brain gene expression in silver foxes. Curr. Biol. 15.

Lindblad-Toh, K., Wade, C.M., Mikkelsen, T.S., Karlsson, E.K., Jaffe, D.B., Kamal, M., Clamp, M., Chang, J.L., Kulbokas, E.J., Zody, M.C., 2005. Genome sequence, comparative analysis and haplotype structure of the domestic dog. Nature 438, 803–819. https://doi.org/10.1038/nature04338

Lindeboom, M., van Doorslaer, E., 2004. Cut-point shift and index shift in self-reported health. J. Health Econ. 23, 1083–1099. https://doi.org/10.1016/J.JHEALECO.2004.01.002

Marder, A., Duxbury, M.M., 2008. Obtaining a Pet: Realistic Expectations. Vet. Clin. North Am. Small Anim. Pract. 38, 1145–1162. https://doi.org/10.1016/j.cvsm.2008.04.011

Marinelli, L., Adamelli, S., Normando, S., Bono, G., 2007. Quality of life of the pet dog: Influence of owner and dog's characteristics. Appl. Anim. Behav. Sci. 108, 143–156. https://doi.org/10.1016/j.applanim.2006.11.018

Martínez, Á.G., Santamarina Pernas, G., Diéguez Casalta, F.J., Suárez Rey, M.L., De la Cruz Palomino, L.F., 2011. Risk factors associated with behavioral problems in dogs. J. Vet. Behav. Clin. Appl. Res. 6, 225–231. https://doi.org/10.1016/j.jveb.2011.01.006

McGarrity, M.E., Sinn, D.L., Thomas, S.G., Marti, C.N., Gosling, S.D., 2016. Comparing the predictive validity of behavioral codings and behavioral ratings in a working-dog breeding program. Appl. Anim. Behav. Sci. 179, 82–94. https://doi.org/10.1016/j.applanim.2016.03.013

McMillan, F.D., Duffy, D.L., Serpell, J.A., 2011. Mental health of dogs formerly used as "breeding stock" in commercial breeding establishments. Appl. Anim. Behav. Sci. https://doi.org/10.1016/j.applanim.2011.09.006

McMillan, F.D., Serpell, J.A., Duffy, D.L., Masaoud, E., Dohoo, I.R., 2013. Differences in behavioral characteristics between dogs obtained as puppies from pet stores and those obtained from noncommercial breeders. J. Am. Vet. Med. Assoc. 242, 1359–1363. https://doi.org/10.2460/javma.242.10.1359

Meaney, M.J., 2001. Maternal care, gene expression, and the transmission of individual differences in stress reactivity across generations. Annu. Rev. Neurosci. 24, 1161–1192. https://doi.org/10.1146/annurev.neuro.24.1.1161

Morrow, M., Ottobre, J., Ottobre, A., Neville, P., St-Pierre, N., Dreschel, N., Pate, J.L., 2015. Breed-dependent differences in the onset of fear-related avoidance behavior in puppies. J. Vet. Behav. 10, 286–294. https://doi.org/10.1016/J.JVEB.2015.03.002

Neumann, I.D., Krömer, S.A., Bosch, O.J., 2005. Effects of psycho-social stress during pregnancy on neuroendocrine and behavioural parameters in lactation depend on the genetically determined stress vulnerability. Psychoneuroendocrinology 30, 791–806. https://doi.org/10.1016/J.PSYNEUEN.2005.03.008

O'Connor, R., Coe, J.B., Niel, L., Jones-Bitton, A., 2016. Effect of Adopters' Lifestyles and Animal-Care Knowledge on Their Expectations Prior to Companion-Animal Guardianship. J. Appl. Anim. Welf. Sci. 19, 157–170. https://doi.org/10.1080/10888705.2015.1125295

Overall, K.L., 2013. Manual of clinical behavioral medicine for dogs and cats. Elsevier Mosby Publishing, St. Louis.

Patronek, G.J., Glickman, L.T., Beck, A.M., McCabe, G.P., Ecker, C., 1996. Risk factors for relinquishment of dogs to an animal shelter. J. Am. Vet. Med. Assoc. 209, 572–81.

Payne, E., Bennett, P., McGreevy, P., 2015. Current perspectives on attachment and bonding in the dog–human dyad. Psychol. Res. Behav. Manag. 71. https://doi.org/10.2147/PRBM.S74972

Piaget, J., 1977. The development of thought : equilibration of cognitive structures. Viking Press, New York.

Pierantoni, L., Albertini, M., Pirrone, F., 2011. Prevalence of owner-reported behaviours in dogs separated from the litter at two different ages. Vet. Rec. 169, 468. https://doi.org/10.1136/vr.d4967

Pirrone, F., Pierantoni, L., Pastorino, G.Q., Albertini, M., 2016. Owner-reported aggressive behavior towards familiar people may be a more prominent occurrence in pet shop-traded dogs. J. Vet. Behav. Clin. Appl. Res. 11, 13–17. https://doi.org/10.1016/j.jveb.2015.11.007

Previde, E.P., Ghirardelli, G., Marshall-Pescini, S., Valsecchi, P., 2009. Intraspecific attachment in domestic puppies (Canis familiaris). J. Vet. Behav. Clin. Appl. Res. 4, 89–90. https://doi.org/10.1016/J.JVEB.2008.10.002

Riemer, S., Müller, C., Virányi, Z., Huber, L., Range, F., 2014. The Predictive Value of Early Behavioural Assessments in Pet Dogs – A Longitudinal Study from Neonates to Adults. PLoS One 9, e101237. https://doi.org/10.1371/journal.pone.0101237

Riemer, S., Müller, C., Virányi, Z., Huber, L., Range, F., 2013. Choice of conflict resolution strategy is linked to sociability in dog puppies. Appl. Anim. Behav. Sci. 149, 36–44. https://doi.org/10.1016/j.applanim.2013.09.006

Rooney, N., Bradshaw, J., Robinson, I., 2000. A comparison of dog–dog and dog–human play behaviour. Appl. Anim. Behav.

Russell, P.A., 1973. RELATIONSHIPS BETWEEN EXPLORATORY BEHAVIOUR AND FEAR: A REVIEW. Br. J. Psychol. 64, 417–433. https://doi.org/10.1111/j.2044-8295.1973.tb01369.x

Salman, M.D., New, J.G., Scarlett, J.M., Kass, P.H., Ruch-Gallie, R., Hetts, S., 2010. Human and Animal Factors Related to the Relinquishment of Dogs and Cats in 12 Selected Animal Shelters in the United States. J. Appl. Anim. Welf. Sci. 1, 207–226.

Scott, J.P., Fuller, J.L., 1965. Genetics and the Social Behavior of the Dog. University of Chicago Press, Chicago.

Shepherd, K., 2009. Behavioural medicine as an integral part of veterinary practice, in: BSAVA Manual of Canine and Feline Behavioural Medicine. British Small Animal Veterinary Association, pp. 10–23. https://doi.org/10.22233/9781905319879.2

Spangenberg, E.M.F., Björklund, L., Dahlborn, K., 2006. Outdoor housing of laboratory dogs: Effects on activity, behaviour and physiology. Appl. Anim. Behav. Sci. 98, 260–276. https://doi.org/10.1016/J.APPLANIM.2005.09.004

STATBEL, 2010. De honden en katten van de Belgen [WWW Document]. URL http://statbel.fgov.be/nl/binaries/De honden en katten van de Belgen_2008_nl_tcm325-103755.pdf

Stepita, M.E., Bain, M.J., Kass, P.H., 2013. Frequency of CPV Infection in Vaccinated Puppies that Attended Puppy Socialization Classes. J. Am. Anim. Hosp. Assoc. 49, 95–100. https://doi.org/10.5326/JAAHA-MS-5825

Svartberg, K., 2002. Shyness–boldness predicts performance in working dogs. Appl. Anim. Behav. Sci. 79, 157–174. https://doi.org/10.1016/S0168-1591(02)00120-X

Tami, G., Gallagher, A., 2009. Description of the behaviour of domestic dog (Canis familiaris) by experienced and inexperienced people. Appl. Anim. Behav. Sci. 120, 159–169. https://doi.org/10.1016/j.applanim.2009.06.009

Titulaer, M., Blackwell, E.J., Mendl, M., Casey, R.A., 2013. Cross sectional study comparing behavioural, cognitive and physiological indicators of welfare between short and long term kennelled domestic dogs. Appl. Anim. Behav. Sci. 147, 149–158. https://doi.org/10.1016/j.applanim.2013.05.001

Turcsán, B., Kubinyi, E., Miklósi, Á., 2011. Trainability and boldness traits differ between dog breed clusters based on conventional breed categories and genetic relatedness. Appl. Anim. Behav. Sci. https://doi.org/10.1016/j.applanim.2011.03.006

van Rooy, D., Arnott, E.R., Early, J.B., McGreevy, P., Wade, C.M., 2014. Holding back the genes: limitations of research into canine behavioural genetics. Canine Genet. Epidemiol. 1, 1–11. https://doi.org/10.1186/2052-6687-1-7

Verbeek, M.E.M., Drent, P.J., Wiepkema, P.R., 1994. Consistent individual differences in early exploratory behaviour of male great tits. Anim. Behav. 48, 1113–1121. https://doi.org/10.1006/ANBE.1994.1344

vonHoldt, B.M., Pollinger, J.P., Lohmueller, K.E., Han, E., Parker, H.G., Quignon, P., Degenhardt, J.D., Boyko, A.R., Earl, D.A., Auton, A., Reynolds, A., Bryc, K., Brisbin, A., Knowles, J.C., Mosher, D.S., Spady, T.C., Elkahloun, A., Geffen, E., Pilot, M., Jedrzejewski, W., Greco, C., Randi, E., Bannasch, D., Wilton, A., Shearman, J., Musiani, M., Cargill, M., Jones, P.G., Qian, Z., Huang, W., Ding, Z.-L., Zhang, Y., Bustamante, C.D., Ostrander, E.A., Novembre, J., Wayne, R.K., 2010. Genome-wide SNP and haplotype analyses reveal a rich history underlying dog domestication. Nature 464, 898–902. https://doi.org/10.1038/nature08837

Wauthier, L.M., Scottish Society for the Prevention of Cruelty to Animals (Scottish SPCA), Williams, J.M., 2018. Using the mini C-BARQ to investigate the effects of puppy farming on dog behaviour. Appl. Anim. Behav. Sci. 206, 75–86. https://doi.org/10.1016/J.APPLANIM.2018.05.024

Weinstock, M., 1997. Does Prenatal Stress Impair Coping and Regulation of Hypothalamic-Pituitary-Adrenal Axis? Neurosci. Biobehav. Rev. 21, 1–10. https://doi.org/https://doi.org/10.1016/S0149-7634(96)00014-0

Weng, H.Y., Kass, P.H., Hart, L.A., Chomel, B.B., 2006. Risk factors for unsuccessful dog ownership: An epidemiologic study in Taiwan. Prev. Vet. Med. 77, 82–95. https://doi.org/10.1016/j.prevetmed.2006.06.004

Wilsson, E., Sundgren, P.-E., 1998. Behaviour test for eight-week old puppies—heritabilities of tested behaviour traits and its correspondence to later behaviour. Appl. Anim. Behav. Sci. 58, 151–162. https://doi.org/http://dx.doi.org/10.1016/S0168-1591(97)00093-2

Wright, J.C., 1983. The effects of differential rearing on exploratory behavior in puppies. Appl. Anim. Ethol. 10, 27–34. https://doi.org/10.1016/0304-3762(83)90109-8

Zhang, T.-Y., Meaney, M.J., 2010. Epigenetics and the Environmental Regulation of the Genome and Its Function. Annu. Rev. Psychol. 61, 439–466. https://doi.org/10.1146/annurev.psych.60.110707.163625

CHAPTER 6:
ASSESSMENT OF BEHAVIOUR AND HEALTH OF PUPPIES AFTER HOMING

This chapter has been adapted from:

From breeding facility to the owners' home:

Health and behavioural quality assessment of puppies.

AUTHORS

Pierre-Alexandre Dendoncker[1,3,4], Christel P.H. Moons[3], Tiny De Keuster[3], Catharina M.M. Lensen[1], Jeroen Dewulf[4], Etienne Thiry[2], Claire Diederich[1]

1 Integrated Veterinary Research Unit (IVRU), Department of Veterinary Medicine, Faculty of Sciences, University of Namur, Rue de Bruxelles 61, 5000 Namur

2 Virology, Department of Infectious and Parasitic Diseases, FARAH Centre, Faculty of Veterinary Medicine, University of Liège, Avenue de Cureghem, 10, 4000 Liège, Belgium

3 Laboratory for Ethology, Department Nutrition, Genetics and Ethology, Faculty of Veterinary Medicine, Ghent University, Heidestraat 19, 9820 Merelbeke

4 Veterinary Epidemiology Unit, Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke

Under revision at Journal of Veterinary Behaviour: Clinical Applications and Research

1. ABSTRACT

The aim of this study was to determine to what extent differences in health and behaviour of puppies might be attributable to the breeder environment. A cross-sectional study of newly acquired puppies was conducted from March 2015 to December 2017 in 20 veterinary practices. Veterinarians performed a systematic behaviour and health assessment of 203 puppies during the first puppy consultation and outcomes were combined with the owner's perception of their dog's behaviour by means of a newly developed puppy C-BARQ questionnaire.

Puppies from dog merchants were more often presented to veterinarians because of illness compared to puppies from occasional breeders (MD=0.3; P<0.01; CI=0.1, 0.6). Puppy age was not significantly associated with any items from the behaviour assessment by the veterinarian. Puppies originating from commercial breeders were scored as less fearful than puppies bred by occasional breeders (MD=0.1; P<0.01; CI=0.0, 0.3), and owners of puppies originating from occasional breeders also scored their puppy higher on stranger-directed aggression (MD=0.2; P=0.04; CI=0.0, 0.4) and fear (MD=0.2; P<0.01; CI=0.2, 0.4), and on nonsocial fear (MD= 0.3; P=0.04; CI=0.0, 0.6) than owners of puppies originating from dog merchants. Puppies from occupational breeders were found to be more passive compared to dogs from occasional breeders (MD= 0.3; P<0.01; CI=0.1, 0.4), commercial breeders (MD=0.5; P<0.01; CI=0.2, 0.7) and merchants (MD=0.4; P<0.01; CI=0.1, 0.6). These results suggest that the breeder category has an effect on behavioural tendencies. More research is needed to determine the influence later in life.

2. INTRODUCTION

Canine behavioural phenotypes result from environmental and genomic interaction, commencing as early as in utero. In research, behavioural phenotyping is often achieved by means of owner-directed questionnaires. The Canine Behavioural Assessment and Research Questionnaire or C-BARQ (Hsu and Serpell, 2003) is globally the most used owner-directed questionnaire for phenotyping of adult dogs (Barnard et al., 2012; Duffy and Serpell, 2012a; Foyer et al., 2014; Nagasawa et al., 2011; Tamimi et al., 2015; van den Berg et al., 2010), provides sub-scores of various traits, and is available i.a. in French, Dutch and English. Other commonly used owner-directed questionnaires focus on one (Konok et al., 2011; Overall et al., 2006; Temesi et al., 2014; Tiira et al., 2016) or a few traits (Ley et al., 2009; van den Berg et al., 2006), or were not translated into languages spoken by our target population (i.e., Dutch and French) (Mirkó et al., 2012).

In humans, behavioural development is the process from birth until adulthood, where psycho-motor and cognitive capacities evolve gradually at a pace and span influenced by the environment (Piaget, 1977). Similar principles apply to dogs (Gómez, 2005), and the importance of the social and nonsocial environment on the behavioural development of dogs has been widely acknowledged (Gazzano et al., 2008; Goddard and Beilharz, 1982; Strandberg et al., 2005; Wilsson and Sundgren, 1998a). Historically, this behavioural ontogeny has been divided into different stages (Overall, 2013; Scott and Fuller, 1965). During the neonatal period (i.e., the first three weeks of life), puppies are in a state of neural immaturity (Scott, 1958). The subsequent socialisation period, from the fourth week up to four months of age, is generally accepted as strongly impacting future adult behaviour (Immelmann and Suomi, 1981; Scott and Fuller, 1965). In parallel, a sensitive period, during which puppies are more able to benefit from exposure to a range of stimuli, has been described as an important age for learning, as providing moderate stress accelerates the maturation of the brain and provides social stability at a later age (Fox, 1971; Fox and Stelzner, 1966).

Previous research on several species has been performed to identify which social and environmental stimuli, or the lack thereof, could cause impairments in behavioural development and functioning (Clarke et al., 1994; Dahlöf et al., 1978; Lee et al., 2007; Takahashi et al., 1992; Tuchscherer et al., 2002). Providing controlled stressors to the offspring on the other hand, such

as gentle handling, and the controlled positive exposure to unfamiliar people, other animals, and novel nonsocial stimuli have been proven to be an effective rearing strategy (Wright, 1983), especially when maternal care is limited (Fox and Stelzner, 1966). While good maternal care is important (Wilsson, 1984), absence of littermates and limitation of environmental resources may also increase the animals' chances of developing future problematic behaviours (Pierantoni et al., 2011; Tiira and Lohi, 2015).

For many years, there has been debate about the link between a dogs' origin and the occurrence of behavioural impairments, dog bite accidents and/or shelter relinquishment. There is growing evidence that behavioural impairments originate at the breeder. In some studies, large-scale breeders such as commercial breeders and pet shops were identified as the source of the problem with regard to improper puppy socialisation (Fatjo et al., 2007; McMillan, 2017; Nagasawa et al., 2016). In other studies, breeding by private persons (i.e., small-scale occasional breeders) was considered a risk factor for shelter relinquishment (Patronek et al., 1996) or for undesirable behaviour such as inappropriate elimination, unwanted chewing (Blackwell et al., 2008), or yelping (Gazzano et al., 2008). These contradictory results may be due to differences in husbandry practices and infrastructure in different countries. However, behavioural development will continue after homing (Riemer et al., 2014), therefore, not only the environment at the breeder, but also the environment at the owner can affect the emergence of future behavioural impairments (Tiira and Lohi, 2015). Undoubtedly, also the health of the animals will influences their behaviour (Frank, 2014). It is not clear if above-mentioned research focused only on breeder influences, ruling out the owners influence on the dogs' behaviour. In our previous study, we found great variation between breeders with regard to housing conditions and procedures, influencing socialisation and environmental learning (Dendoncker et al., 2019). Considering the current social debate on the impact of the breeder type on puppy health and behaviour, the aim of this study was to determine to what extent differences in health and behaviour of puppies, in early stages after acquisition, might be attributable only to the breeder environment.

3. MATERIALS AND METHODS

3.1. Study design

A cross-sectional study of newly acquired puppies was conducted from March 2015 to December 2017 at 20 Belgian veterinary practices. For this study, veterinarians were recruited by means of contacting practices directly, through advertorials in veterinary journals, or by giving short presentations during events for veterinarians. Participating veterinarians invited clients visiting with puppies to join the study. If clients agreed, data were recorded on the identification of the dog, owner, and breeder, on medical history, and on the reason for the consultation. A behavioural assessment and physical examination of puppies were performed in-clinic by the veterinarian and were encoded in an internet survey tool (Limesurvey®). Furthermore, owners were asked to complete an online or paper questionnaire, which was a modified version of the C-BARQ, about the behaviour of their puppy. To describe the acquisition sources of pet dogs we used the four dog breeder categories based on the observations during our previous study (Dendoncker et al., 2018). These categories hinge on the number of dams and the number of puppies sold yearly and revealed to be different in education, husbandry management and practices relevant to behavioural development of puppies (Dendoncker et al., 2019). A detailed description of the breeder classification can be found in chapter 3.

3.2. Data collection

Participating veterinarians were asked to complete an online training on behavioural assessment of pet dogs and an online performance check before commencing data collection. Training consisted of rating videos and photographs depicting four canine behavioural traits: fearful, aggressive, neutral and playful (Lensen et al., 2013b), which was developed by Lensen et al., (2013a).

Veterinarians used a standardised questionnaire to score puppies' behaviour and health during the physical examination. The full protocol, hereafter called Behavioural Assessment and Screening of Health (BASH), took into account certain constraints of a puppy consultation, such as the relatively short timeframe and limited availability of tools. The occurrence of behaviours was recorded by indicating whether a behaviour was absent (0%), less common (1-80%), or

more common (>80% of the time) (Lensen et al., 2014).The health check-sheet (29 items) was developed based on literature about the canine paediatric physical examination (Hoskins and Partington, 2001; Nagle, 2006; Root Kustritz, 2011) and was pretested by nine veterinarians not participating in the study. Particular attention was given to the presence of infectious agents or congenital defects by listing the most common ones in the questionnaire. Veterinarians could easily and quickly select the involved system (e.g., skin), and the dynamic questionnaire added additional questions (e.g., if the presence of ecto-parasites was confirmed, a drop-down appeared which listed the parasites with high prevalences, such as fleas). Numerical responses were chosen when possible, but veterinarians also had the option to comment freely on each question. The behavioural assessment is available as supplementary material (supplementary file 7.1).

The original C-BARQ consists of 101 items (Hsu and Serpell, 2003), and a reduced version of 42 items exists for shelter dogs (Duffy et al., 2014). However, none of these versions have been developed for use in puppies. In order to make the questionnaire suitable for puppy behavioural assessment, C-BARQ was modified according to the Delphi Technique (Dalkey and Helmer, 1963). Adaptations to a questionnaire by means of this expert elicitation method will preserve content validity when the panels participating in the study are representative of the research field (Goodman, 1987). First, a behavioural expert panel (n=10) evaluated if the original 101 items were appropriate for puppies, by means of an online survey. Forty-four questions were deemed less applicable for puppies by at least two experts (for example: question n°23: *When approached directly by an unfamiliar male dog while being walked/exercised on a leash*). These questions were then modified based on the experts' suggestions (question n°23 adapted: *When approached directly by an unfamiliar adult dog*). Subsequently, a second evaluation round of the modified questions was performed by the expert panel. Final evaluation resulted in the removal of two questions, modification of seven questions, and the merging of nine questions. In addition, the answer option 'not applicable' was introduced, as owners of puppies might not yet have encountered some of the situations presented in the C-BARQ. This *puppy C-BARQ* (Dendoncker et al., 2015) consists of 89 items and is available as supplementary material (supplementary file 7.2).

3.3. Statistical analysis

Descriptive and inferential statistical analyses were performed using SPSS 24 (IBM SPSS ®). Normality was assessed for all continuous variables (e.g., age, duration of ownership). Because the median is a reliable indicator in highly skewed C-BARQ scores (Dodman et al., 2018; Nagasawa et al., 2012), and because the data had mainly a bi-modal distribution, we dichotomised the scores by considering the median as cut-off value (Dalla Villa et al., 2017) This approach provides a relative measure of a dog's behaviour (e.g., aggressiveness) to the average behaviour of the total sample (Hsu and Sun, 2010). Items of the health screening that had not been performed on every dog (e.g., laryngo-tracheal reflex was only performed on 79 puppies) were removed from the analysis. Data from the behavioural assessment were recoded into binomial values (i.e., dog showing the behaviour / not showing the behaviour). The *general behavioural impression* of the dog during the behaviour assessment was recoded into four variables (fearful, aggressive, neutral, and playful).

Continuous variables were analysed by performing a one-way ANOVA with post-hoc Tukey's honestly significant difference test to correct for multiple comparisons. Binomial variables (i.e., results of puppy C-BARQ and BASH) were analysed using binary logistic regression by means of generalised linear models with Šidák correction for multiple comparisons. We provided the effect size as Eta Squared (η^2) and, the F-Value (F), degrees of freedom (dF) and the statistical significance (corrected P-value) when possible for each continuous variable and we provided the effect size as mean difference (MD), corrected P-Value and Confidence Interval (CI) for each binomial variable. We compared the effects of breeder type, veterinarian, breed, breed group, duration of ownership (in days), sex and age of puppies (in days). Agreement between the BASH and puppy C-BARQ was assessed by means of Cohen's kappa.

4. RESULTS

Thirty-eight veterinarians completed the online training and 20 collected *full data* (data from both BASH and puppy C-BARQ) In total, 219 puppies (range: 1 to 26 puppies per veterinarian) from various breeds and aged 38 to 188 days old (mean 91.1, SD 32.3) were assessed during their first visit to the veterinary practice after rehoming. Sixteen puppies were excluded because of uncertain origin. The data distribution across breeder categories is shown in table 6.1. 131 puppies (64.5%) had *full data* whereas 72 only had data from the veterinarian. The sample of puppies was representative when compared to governmental data (ABIEC-BVIRH, Unpublished data) for source of origin (i.e., the Belgian classification), breed and sex (51.4% females). None of the puppies were neutered. Breeder categories did not differ significantly for breed (P=0.4) or sex (P=0.5).

Table 6.1: Number of puppies assessed by BASH and/or Puppy C-BARQ per breeder category

	Small-scale breeders		Large-scale breeders		
	Occasional	Occupational	Professional	Merchant	Total:
BASH + Puppy C-BARQ	70	14	22	25	131
Only BASH	24	12	22	14	72
Total	94	26	44	39	203

Table 6.2 lists the age of puppies (in days) during the physical examination, and duration of ownership (in days). The main foreign countries of origin were Slovakia (31), The Netherlands (7) and Czech Republic (5). Puppies from foreign origin were significantly older ($F_{1,127}$=26.5; P<0.01; η^2=0.2) and were more often sold by large-scale breeders. Merchants sold puppies from foreign origin more often than occasional breeders (MD=0.4; P<0.01; CI=0.2, 0.6), occupational breeders (MD=0.5; P<0.01; CI=0.3, 0.7) and commercial breeders (MD=0.3; P=0.02; CI=0.2, 0.6) and commercial breeders sold puppies from foreign origin more often than occupational breeders (MD=0.2; P=0.03; CI=0.0, 0.3). The duration of ownership (range 1 to 127 days) did not differ significantly ($F_{3,127}$=1.5; P=0.2; η^2=0.04) between breeder categories.

Table 6.2: Puppy age (in days), ownership duration (in days) and country of origin of the puppies for each breeder category.

	Overall statistical effect (P-value)	Small-scale Breeders		Large-scale Breeders	
		Occasional breeder[a,b] (n=70)	Occupational breeder (n=14)	Professional breeder (n=22)	Merchant (n=25)
Puppy age (SD)	<0.01	84.89[a] (26.57)	77.29[b] (22.20)	100.18[a,c] (38.91)	114.44[c] (35.26)
Ownership duration (SD)	0.2	26.01[a] (26.72)	10.79[a] (13.29)	18.00[a] (20.94)	22.68[a] (34.91)
Belgian origin	<0.01	95.2%[a]	100.0%[a]	81.8%[b]	52.0%[c]

[a-b] Different indexes indicate a significant difference (P≤0.05) between categories.

4.1. Behavioural assessment and screening of health

Results from the behavioural assessment by veterinarians are listed in tables 6.3 and 6.4. Dogs from small-scale breeders were more reluctant to enter the examination room (MD=0.2; P=0.01; CI=0.0, 0.4) and more looking for support from the owner (i.e., attention-seeking behaviour) (MD=0.2; P=0.02; CI=0.0, 0.4) compared to the ones from large-scale breeders.

Dogs from commercial breeders were less reluctant to enter the examination room (MD=0.3; P=0.04; CI=0.0, 0.5) and they were less fearful (MD=0.1; P<0.01; CI=0.0, 0.3) compared to dogs from occasional breeders. They were also scored as more active (MD=0.4; P=0.04; CI=0.0, 0.8) than dogs from occupational breeders. Dogs from occupational breeders showed less resistance to manipulation by the veterinarian compared to dogs of other categories. They showed less resistance compared to the ones from occasional breeders (MD= 0.3; P<0.01; CI=0.1, 0.4), commercial breeders (MD=0.5; P<0.01; CI=0.2, 0.7) and merchants (MD=0.4; P<0.01; CI=0.1, 0.6).

No significant differences in owner behaviour were found between breeder categories. Veterinarians found owners of older dogs to be controlling more often ($F_{1,126}$=5.8; P=0.02; η^2=0.04). Age was not significantly associated with other items of the behavioural assessment by veterinarians (P>0.07), nor was the duration of ownership (P>0.05). Behaviour was not significantly different for puppies of foreign origin (P>0.05).

Table 6.3: Proportion (%) of puppies that perform a particular behaviour in veterinary clinic.

	Overall statistical effect (P-value)	Small-scale breeders		Large-scale breeders	
		Occasional breeder (n=94)	Occupational breeder (n=26)	Professional breeder (n=44)	Merchant (n=39)
Reluctant to enter	0.02	18.2[a]	36.4[a,b]	5.7[b]	7.7[a,b]
Reluctant to exit	0.09	10.4[a]	14.3[a]	8.1[a]	3.3[a]
Fearful	<0.01	11.7[a]	15.4[a,b]	4.5[b]	18.4[a,b]
Aggressive	0.2	4.3[a]	0.0[a]	0.0[a]	2.6[a]
Neutral	0.4	42.6[a]	61.5[a]	52.3[a]	50.0[a]
Playful	0.09	40.4[a]	15.4[a]	38.6[a]	28.9[a]
Resistance to manipulation	<0.01	25.0[a]	3.8[b]	33.3[a]	31.6[a]
Avoidance to manipulation	0.2	31.9[a]	20.0[a]	35.7[a]	24.3[a]
Stiff posture	0.7	13.0[a]	4.0[a]	14.6[a]	16.2[a]
Low posture	0.5	27.2[a]	40.0[a]	16.7[a]	21.6[a]
Panting	0.1	24.2[a]	12.0[a]	28.6[a]	37.8[a]
Wailing	0.9	23.7[a]	24.0[a]	26.2[a]	13.9[a]
High activity	0.04	77.2[a,b]	48.0[a]	72.1[b]	75.7[a,b]
Looking for Support	0.03	64.5[a]	44.0[a,b]	47.6[b]	51.4[a,b]
Social towards vet	0.8	90.3[a]	76.0[a]	85.4[a]	78.4[a]
Stressed	0.4	38.0[a]	24.0[a]	35.7[a]	42.1[a]

[a-b] Different indexes indicate a significant difference (P≤0.05) between categories.

Table 6.4: Proportion (%) of owners that perform a particular behaviour in veterinary clinic.

	Overall statistical effect (P-value)	Small-scale breeders		Large-scale breeders	
		Occasional breeder (n=94)	Occupational breeder (n=26)	Professional breeder (n=44)	Merchant (n=39)
Owner nervous	0.3	22.2[a]	26.9[a]	28.6[a]	39.5[a]
Owner indifferent	0.4	10.0[a]	8.0[a]	14.3[a]	5.4[a]
Owner manipulative	0.9	92.3[a]	96.0[a]	85.7[a]	89.2[a]
Owner controlling	0.3	34.1[a]	25.0[a]	26.2[a]	18.9[a]
Owner reassuring	0.9	81.5[a]	76.0[a]	71.4[a]	78.4[a]

[a] Different indexes indicate a significant difference (P≤0.05) between categories.

Tables 6.5 and 6.6 list the health screening results. Puppies taken to the veterinary clinic because of an illness (instead of a health check-up) originated more often from large-scale breeders compared to small-scale breeders (MD=0.3; P<0.01; CI=0.1, 0.4) and especially from merchants compared to occasional breeders (MD=0.3; P<0.01; CI=0.1, 0.6). Puppies presented due to illness were not older ($F_{1,136}$=1.9; P=0.2; η^2=0.01) nor had a longer duration of ownership ($F_{1,136}$=0.2; P=0.6; η^2<0.01). 119 puppies (54.3%) were presented with at least one problematic finding in the health check-up (mean 1.2; SD ±1.5), regardless of breeder category (MD=0.1; P=0.4; CI=-0.2, 0.1). The most common findings were skin disorder (i.e., flea infestation) (n=37), gastro-intestinal disorder (e.g., dilated abdomen, diarrhoea) (n=30), overweight (n=25), eye disorder (i.e., epiphora) (n=23), underweight (n=22), ear disorder (n=21), nose disorder (i.e., stridor) (n=17), abnormal respiration (n=15), abnormal pulmonary auscultation (n=14).

Dogs from merchant were more often overweight (MD=0.3; P=0.05; CI=0.0, 0.5) compared to the ones from occasional breeders. The age was not significantly higher for overweight puppies ($F_{1,132}$=0.6; P=0.4; η^2=0.04) nor was the duration of ownership longer ($F_{1,132}$=2.9; P=0.09; η^2=0.02). Puppies from foreign origin presented respiratory symptoms more often (MD=0.2; P=0.02; CI=0.0, 0.4), but the origin had also a significant effect ($F_{1,132}$=4.0; P=0.05; η^2=0.03). Additionally, a discrepancy between age stated on the passport and observed teething was not significantly associated with origin (MD=0; P=0.4; CI=-0.1, 0.1), nor was country of origin associated with higher prevalence of other health disorders (MD=0; P=0.9; CI=-0.2, 0.2).

Primo-vaccination with core vaccines (CPV, CDV, Canine Adenovirus (CAV), and rabies when required by statue), such as recommended by the World Small Animal Veterinary Association (WSAVA) guidelines, was well applied. Large-scale breeders did not differ in the implementation thereof from small-scale breeders (MD=0; P=0.4; CI=0.0, 0.1). Deworming protocols were most often not provided or unknown to the owner and no difference between breeders could be demonstrated.

Table 6.5: Proportion (%) of puppies per breeder presented with a particular medical history (n=203)

	Overall statistical effect (P-value)	Small-scale breeders		Large-scale breeders	
		Occasional breeder (n=94)	Occupational breeder (n=26)	Professional breeder (n=44)	Merchant (n=39)
Reason of consultation is illness	<0.01	2.1[a]	3.8[a,b]	18.2[a,b]	33.3[b]
History of previous illness	0.2	8.6[a]	0.0[a]	9.1[a]	8.0[a]
Primo-vaccine following guidelines[1]	0.3	91.5[a]	88.5[a]	90.0[a]	97.4[a]
Dewormed at least once	0.4	60.6[a]	61.5[a]	56.8[a]	48.7[a]
Dewormed following guidelines[2]	0.4	25.5[a]	30.8[a]	15.9[a]	17.9[a]

[a-b] Different indexes indicate a significant difference (P≤0.05) between categories.

[1]Guidelines for vaccination provided by WSAVA (2016)

[2]Guidelines for deworming provided by ESCCAP (2010)

Table 6.6: Proportion (%) of puppies per breeder category with a specific symptom as examined during the health screening (n=203)

	Overall statistical effect (P-value)	Small-scale breeders		Large-scale breeders	
		Occasional breeder (n=94)	Occupational breeder (n=26)	Professional breeder (n=44)	Merchant (n=39)
Abnormality on physical examination during check-up	0.5	34.3[a]	21.4[a]	22.7[a]	32.0[a]
Teething different from passport's age	0.5	4.3[a]	11.5[a]	6.8[a]	10.3[a]
Abnormal body temperature	0.8	18.1[a]	24.0[a]	16.7[a]	35.1[a]
Abnormal breathing	0.07	6.4[a]	0.0[a]	6.8[a]	10.3[a]
Abnormal cardiac auscultation	0.6	1.1[a]	0.0[a]	2.3[a]	0.0[a]
Abnormal pulmonary auscultation	0.2	3.3[a]	7.7[a]	9.1[a]	13.5[a]
Lesions or congenital eye defects	0.9	9.6[a]	7.7[a]	13.6[a]	10.3[a]
Lesions or congenital nose defects	0.5	8.5[a]	15.4[a]	4.5[a]	5.1[a]
Lesions or congenital buccal defects	0.6	5.3[a]	15.4[a]	4.5[a]	5.1[a]
Lesions or congenital ear defects	0.5	9.6[a]	3.8[a]	11.4[a]	10.3[a]
Lesions or congenital limb defects	0.3	1.1[a]	11.5[a]	4.5[a]	2.6[a]
Lesions or congenital urogenital defects	0.7	2.1[a]	3.8[a]	6.8[a]	2.6[a]
Lesions or congenital anal defects	0.06	4.3[a]	3.8[a]	4.5[a]	0.0[a]
Lesions or congenital tail defects	0.9	4.3[a]	3.8[a]	2.3[a]	2.6[a]
Presence of dermatologic disorder	0.4	22.3[a]	15.4[a]	11.4[a]	15.4[a]
Presence of ectoparasites	0.9	14.9[a]	11.5[a]	11.4[a]	10.3[a]
Abnormal abdominal palpation	0.7	12.8[a]	23.1[a]	11.4[a]	12.8[a]
Underweight	0.4	11.7[a]	12.0[a]	4.8[a]	10.8[a]
Overweight	0.1	6.4[a]	12.0[a,b]	11.9[a,b]	24.3[b]

[a-b] Different indexes indicate a significant difference (P≤0.05) between categories.

4.2. Puppy behavioural assessment

The score for each puppy C-BARQ behavioural category is available as supplementary material (Supplementary file 7.3).

Table 6.7 lists the proportion of puppies with a score above the median (cut-off value) for each puppy C-BARQ category. Dogs from large-scale breeders scored significantly lower on stranger-directed aggression (MD=0.2; P=0.01; CI=0.0, 0.3) and stranger-directed fear (MD=0.2; P=0.03; CI=0.0, 0.3). Specifically, dogs of merchants scored lower on stranger-directed aggression compared to dogs from occasional (MD=0.2; P=0.04; CI=0.0, 0.4) and occupational breeders (MD=0.5; P=0.01; CI=0.0, 0.9), and scored lower on stranger-directed fear (MD=0.2; P<0.01; CI=0.2, 0.4) and nonsocial fear (MD= 0.3; P=0.04; CI=0.0, 0.6) compared to occasional breeders. No other puppy C-BARQ traits were found to be significantly different between small-scale and large-scale breeders. Higher puppy C-BARQ scores for excitability ($F_{1,136}$=4.9; P=0.03; η^2=0.04) and energy level ($F_{1,135}$=4.0; P=0.05; η^2=0.03) were observed with increasing duration of ownership. Age was not significantly associated with puppy C-BARQ traits (P>0.05) nor was a foreign origin (P>0.05).

4.3. Association of assessment by the owner and by a veterinarian

We compared the assessment of the puppy by the owner (puppy C-BARQ) with the behavioural assessment by the veterinarian (BASH). Similar traits of Puppy C-BARQ and BASH presented a low level of agreement (κ<0.2).

Table 6.7: Proportion (%) of puppies (n=131) for each breeder category with a puppy C-BARQ score above median.

	Overall statistical effect (P-value)	Small-scale breeders		Large-scale breeders	
		Occasional breeder (n=70)	Occupational breeder (n=14)	Professional breeder (n=22)	Merchant (n=25)
Stranger-directed aggression	<0.01	30.0[a]	46.2[a]	22.7[a,b]	8.3[b]
Owner-directed aggression	0.8	24.3[a]	21.4[a]	27.3[a]	16.7[a]
Dog-directed aggression	0.2	35.9[a]	16.7[a]	42.9[a]	22.7[a]
Stranger-directed fear	<0.01	27.9[a]	28.6[a,b]	22.7[a,b]	4.2[b]
Nonsocial fear	0.05	48.6[a]	42.9[a,b]	45.5[a,b]	20.8[b]
Dog-directed Fear	0.2	44.6[a]	18.2[a]	30.0[a]	34.8[a]
Separation-related behavior	0.2	38.6[a]	21.4[a]	40.9[a]	54.2[a]
Attachment and attention seeking	0.3	54.3[a]	64.3[a]	40.9[a]	33.3[a]
Trainability	0.4	57.1[a]	35.7[a]	45.5[a]	44.0[a]
Chasing	0.6	40.9[a]	42.9[a]	30.0[a]	28.0[a]
Excitability	0.3	50.0[a]	28.6[a]	45.5[a]	36.0[a]
Touch sensitivity	0.4	40.6[a]	35.7[a]	40.9[a]	24.0[a]
Energy level	0.8	37.1[a]	50.0[a]	36.4[a]	66.7[a]
Dog rivalry	0.5	19.4[a]	40.0[a]	30.0[a]	10.0[a]

[a-b] Different indexes indicate a significant difference (P≤0.05) between categories.

5. DISCUSSION

This study aimed to describe differences in behaviour and health of puppies in the early stages after acquisition, so as to avoid owner-related influences as much as possible. Additionally, this article presents the development and use of tools to achieve the aim, namely a modified C-BARQ or puppy C-BARQ (Dendoncker et al., 2015), in parallel with a behavioural assessment (Lensen et al., 2014) and screening of health (BASH) performed by veterinarians.

Three differences between breeder types were identified when analysing items of behaviour and health. Firstly, dogs from occasional breeders were more fearful, more reluctant to enter the examination room and more looking for support compared to dogs from commercial breeders.

Most likely, these traits identify an underlying emotion: fear towards an unfamiliar environment, such as a veterinary practice (Döring et al., 2009). Earlier findings suggest that puppies raised in family homes (i.e., occasional breeders) show an emotional state that is more easily altered by isolation. Absence of familiar environmental references could generate more stress in those puppies compared to commercially bred puppies (Gazzano et al., 2008). Secondly, puppies from occupational breeders displayed less resistance to manipulation by the veterinarian in comparison to puppies from other categories. They were also scored to be less active and playful during the physical exam than dogs from occasional breeders. Research showed that high activity level of dogs is potentially linked to their performance in specific tasks (Svartberg, 2002; Weiss and Greenberg, 1997). Activity is also recognised as a sign of emotional level (Fox, 1972) and is related the coping style (i.e., proactive or reactive) (Koolhaas et al., 1999) and to future social tendencies (Beaudet et al., 1994). Hence, dogs from occupational breeders possibly were more reactive. Third, owners of older dogs were more often scored as overly controlling. It can be hypothesised that owners expect more obedience with increasing age and experience of their dog. This could also be explained by the performed training technique, as positive punishment is still ubiquitous (Blackwell et al., 2008). For the other items of the behavioural assessment, age of the puppies was not associated significantly, nor were the duration of ownership or the country of origin.

This study indicates that puppies from large-scale breeders were more frequently presented to the veterinarian because of illness rather than for a health check-up and vaccination. The reason for the consultation (i.e., health check-up or illness) is important since there is evidence that this influences the veterinarian-client communication and the amount of advice given to the owner (Shaw et al., 2008). Large-scale breeders are often linked to higher prevalence of diseases (Dupont et al., 2013; Hird et al., 1992; Scarlett et al., 1994). In this study we found no such association, but this could be a result of the relatively small sample size. The prevalence of abnormalities found in this study was comparable to a previous larger-scale epidemiological study (Lund et al., 1999).

This study also demonstrates that puppies from large-scale breeders were more frequently presented to the veterinarian at a later age. This could be mainly due to legal regulation: merchants may only trade dogs originating from abroad from the age of 15 weeks (EU

regulation 576/2013). It can be hypothesised that for puppies sold at 16 weeks of age or later, their age coincides with the end of the recommended vaccination protocol and associated health check-ups (Day et al., 2016). Anecdotal evidence and reports from veterinarians in the field point out the possibility of fraud when it concerns age, implying foreign dogs to be younger than 15 weeks. Therefore, veterinarians were asked to check the teething status and to provide the declared age on the passport. However, we found no significant discrepancy between teething status and declared age.

Dogs from small-scale breeders scored significantly higher for stranger-directed aggression and stranger-directed fear. Specifically, puppies from merchants scored lower for stranger-directed aggression compared to occasional and occupational breeders and lower for stranger-directed fear and nonsocial fear compared to occasional breeders. As mentioned earlier, puppies raised in family homes could have an inferior ability to adapt in the absence of familiar environmental references (Gazzano et al., 2008). Certainly, puppies that originate from merchants are more often exposed to strangers, possibly resulting in more habituation or, on the contrary, sensitisation: they move from the original breeder to the collection centre and subsequently to the merchant. Frequently, they undergo long-distance travel, are handled by many different people, and are often presented in a showroom (Dendoncker et al., 2019). Higher scores of excitability and energy level were found with increasing duration of ownership. Long-term owned puppies, that are more likely to be attached to their owners, could be comforted by the owner's presence. Less dog-human bonding is to be expected in short duration of ownership and a higher dog-owner attachment is associated with high energy level (Kurdek, 2008).

This study shows that veterinary practitioners can be of great help to broaden data collected through owner questionnaires. The BASH was intended as a fast and easily applicable tool for practicing veterinarians. While remarks made by participating veterinarians were surveyed, none were directed towards the practicality of BASH. On the contrary, the puppy C-BARQ was considered as a limiting factor in sample size, since the number of items was the first reason not to participate. Owner questionnaires such as the above presented puppy C-BARQ are often used in research. They are quick and cheap tools for repeatable and reliable behavioural phenotyping (Barnard et al., 2012; Duffy and Serpell, 2012b; Foyer et al., 2014; Nagasawa et al., 2011; Tamimi et al., 2015; van den Berg et al., 2010) and have already been used to

compare source of origin (McMillan et al., 2013; Pierantoni et al., 2011). However, since there is evidence of differences between owners of different breeder categories (Pirrone et al., 2016, 2015), one can hypothesise that a comparison of breeders (and thus indirectly a comparison of owners) cannot rely solely on data from owner questionnaires. Puppy C-BARQ and BASH seemed to describe similar variables and showed a likely magnitude in values. The agreement did not differ greatly from chance, meaning methodological differences between the two tests may provide complementary information. The low agreement between both tests may also be the result of different settings (Godbout et al., 2007; Mariti et al., 2015) and different raters (owner versus veterinarian) (Jones and Gosling, 2005).

This study had four limitations. First, during this study we relied on two tools untested for their external validity. These tools allowed us to assess puppy behaviour on two levels: by a veterinary practitioner and by the owner. For the former, we tried to eliminate multi-observer bias (Landis and Koch, 1977) by giving an online training to participating veterinarians and limiting the number of possible answers (Lensen et al., 2013a). We also did not have a gold standard at our disposal for validation of the BASH (Diederich and Giffroy, 2006). For the latter, an additional validation against another questionnaire or in another population was not feasible because of logistic and financial limitations. Also, owner-directed questionnaires are subjected to several biasses (see chapter 5 for an overview). Second, no follow-up was performed and behavioural differences in veterinary practices were measured at one point in time only. the importance of a sensitive period for socialisation in young puppies is often stressed, this does not imply that environmental influences that occur at other developmental stages do not have effects as well (Riemer et al., 2014). Testing of juveniles has been the object of controversy (Wilsson and Sundgren, 1998b), Although many studies have not able to demonstrate a personality consistency in dogs, a recent meta-analysis of these published studies has demonstrated that a certain consistency of personality exists throughout the dog's life (Fratkin et al., 2013). Early observations of puppies, therefore, may provide some valuable information concerning behavioural tendencies and coping style. Nevertheless, it remains advisable to interpret results of early testing of juveniles with caution. Personality consistency is lower in puppies and increases with age. While the importance of a sensitive period for socialisation in young puppies is often stressed, this does not imply that environmental influences that occur at

other developmental stages do not have effects as well (Riemer et al., 2014). Testing young puppies, therefore, can provide some valuable information concerning future behaviour while limiting owner-related effects but does not necessarily predicts the exact adult behaviour. Third, the age of interest in this study also covered the onset of the first fear period (Morrow et al., 2015). This critical stage during socialisation is generally accepted to occur for about one week between the ages of 8-12 weeks (Coppinger and Coppinger, 2001). This could have influenced the level of fear observed during this one time point in puppies. Finally, the study could be subject to selection bias: Only puppies presented to veterinary practices were included. Considering that a large number of owners associates veterinary care primarily with vaccination, or take their dog to a veterinary practice less than once a year (Volk et al., 2011), this limitation could be avoided by repeating this study at breeding facilities or by sampling puppies through registration databases with mandatory enrollment.

6. CONCLUSION

Puppies of foreign origin are more often presented to veterinarians because of illness. However, no differences in occurrence of disorders could be found between Belgian and foreign puppies. Although these preliminary results should be viewed with caution, puppies from large-scale breeders were scored less fearful than puppies from small-scale breeders, not only when taken into a veterinary clinic setting but also as scored at home by their owners. Since studies investigating behaviour of adult dogs reported more fearfulness for dogs from large-scale breeders, it remains uncertain if these differences originate at the breeder or subsequent to later experiences with the owner. Therefore, more research is necessary to investigate different owner types and their influence on the behaviour of their dog.

7. SUPPLEMENTARY MATERIALS

7.1. Behavioural assessment used in veterinary practice

7.1.1. Entry into and departure from the consultation room (one answer per column)

	Entry	**Departure**
Refuses to walk	[ ]	[ ]
Walks hesitantly	[ ]	[ ]
Walks confidently	[ ]	[ ]
Pulls at the leash	[ ]	[ ]

7.1.2. General evaluation of the dog during the clinical examination.

( ) Anxious

( ) Aggressive

( ) Neutral

( ) Playful

( ) Other, _____

7.1.3. Specific evaluation of the behaviour during the clinical examination (from the first manipulation).

	absent	**intermittent**	**Permanent (>80% of time)**
Resistance to manipulation	( )	( )	( )
Avoidance of the veterinarian	( )	( )	( )
Stiff posture	( )	( )	( )
Low posture	( )	( )	( )
Panting	( )	( )	( )

7.1.4. Evaluation whining/ Barking during the clinical examination

( ) Not at all

( ) A few times

( ) Constantly (minimally once every 10 seconds)

7.1.5. General (subjective) evaluation of the dog during the clinical examination

(0 =not at all, 2= extremely):

	0	1	2
Active / Energetic	( )	( )	( )
Looking for support	( )	( )	( )
Social towards the veterinarian	( )	( )	( )
Stressed (intensity)	( )	( )	( )

7.1.6. Do you think that this dog is at higher risk compared to other dogs to develop behavioural impairments?

( ) Yes, what and why do you expect it?_____

( ) No, why don't you expect it?_____

7.1.7. Did you advise your client how to preclude/decrease these possible impairments?

( ) Yes ( ) No

7.1.8. Behaviour of the owner (0 =not at all, 2 = extremely):

	0	1	2
Nervous	( )	( )	( )
Indifferent	( )	( )	( )
Manipulative/ contact towards the dog	( )	( )	( )
Controlling	( )	( )	( )
Comforting	( )	( )	( )

7.2. puppy C-BARQ as provided to dog owners

SECTION 1: *Training and obedience*

Some dogs are more obedient and trainable than others. By checking the appropriate boxes, please indicate how trainable or obedient your puppy has been in each of the following situations in the recent past:

	Never	Seldom	Sometimes	Usually	Always	Not Appl.
When off the leash, returns immediately when called.	O	O	O	O	O	O
Responds to a command most of the time (e.g.,, "sit", stay", come here...).	O	O	O	O	O	O
Seems to attend/listen closely to everything you say or do.	O	O	O	O	O	O
Slow to respond to a correction or punishment (e.g.,, "no", "bad dog", shout at puppy...).	O	O	O	O	O	O
Slow to learn new tricks or tasks.	O	O	O	O	O	O
Easily distracted by interesting sights, sounds or smells.	O	O	O	O	O	O
Will 'fetch' or attempt to fetch sticks, balls, or objects.	O	O	O	O	O	O

SECTION 2: *Aggression*

Some dogs display aggressive behaviour from time to time. Typical signs of moderate aggression in dogs include barking, growling and baring teeth. More serious aggression generally includes following behaviour: snapping, lunging, biting, or attempting to bite.

No aggression:	**Moderate aggression:**	**Serious aggression:**
No visible signs of aggression	Growling/ barking—baring teeth **0.....1.....2.....3.....4**	Snaps, bites or attempts to bite.

By circling or underlining a number on the following 5-point scales (0= No aggression, 4= Serious aggression), please indicate your own puppy's recent tendency to display aggressive behaviour in each of the following contexts:

	0	1	2	3	4	Not Appl.
When verbally corrected or punished (e.g.,, scolded, shouted at...) by you or a household member.	O	O	O	O	O	O
When approached directly by an unfamiliar adult while being walked/exercised on a leash.	O	O	O	O	O	O

When approached directly by an unfamiliar child while being walked/exercised on a leash.	O	O	O	O	O	O
Toward unfamiliar persons approaching the puppy while s/he is in your car (at the petrol station for example).	O	O	O	O	O	O
When toys, bones or other objects are taken away by a household member.	O	O	O	O	O	O
When manipulated by a household member (e.g.,, bathing, grooming, toweling feet, looking at ears...)	O	O	O	O	O	O
When an unfamiliar person approaches you or another member of your family at home.	O	O	O	O	O	O
When unfamiliar persons approach you or another member of your family away from your home.	O	O	O	O	O	O
When approached directly by a household member while s/he (the puppy) is eating.	O	O	O	O	O	O
When postmen or other delivery workers approach your home.	O	O	O	O	O	O
When his/her food is taken away by a household member.	O	O	O	O	O	O
When strangers walk past your home while your puppy is outside or in the garden.	O	O	O	O	O	O
When an unfamiliar person tries to touch or pet the puppy.	O	O	O	O	O	O
When people outside pass at high speed along to the puppy (ex. Jogger, cyclist, skateboard...)	O	O	O	O	O	O
When approached directly by an unfamiliar dog while being walked/exercised on a leash.	O	O	O	O	O	O
When stared at directly by a member of the household.	O	O	O	O	O	O
Toward unfamiliar dogs visiting your home.	O	O	O	O	O	O
Toward cats, squirrels or other small animals entering your garden.	O	O	O	O	O	O
Toward unfamiliar persons visiting your home.	O	O	O	O	O	O
When barked, growled, or lunged at by another (unfamiliar) dog.	O	O	O	O	O	O
When stepped over by a member of the household.	O	O	O	O	O	O
When you or a household member retrieves food or objects stolen by the puppy.	O	O	O	O	O	O
Towards another (familiar) dog in your household. (Leave blank if no other dogs)	O	O	O	O	O	O
When approached at a favorite resting/sleeping place by another (familiar) household dog. (Leave blank if no other dogs)	O	O	O	O	O	O
When approached while eating by another (familiar) household dog. (leave blank if no other dogs)	O	O	O	O	O	O
When approached while playing with/chewing a favorite toy, bone, object... by another (familiar) household dog. (leave blank if no other dogs)	O	O	O	O	O	O

SECTION 3: *Fear and Anxiety*

Dogs sometimes show signs of anxiety or fear when exposed to particular sounds, objects, persons or situations. Typical signs of mild to moderate fear include: avoiding eye contact, avoidance of the feared object; crouching or cringing with tail lowered or tucked between the legs; whimpering or whining, freezing, and shaking or trembling. Extreme fear is characterised by exaggerated cowering and/or vigorous attempts to escape, retreat or hide from the feared object, person or situation.

No fear/ anxiety:	**Mild—Moderate fear/anxiety:**	**Extreme fear:**
No visible signs of fear	**0.....1.....2.....3.....4**	Cowers; retreats or hides, etc.

Using the following 5-point scales (0=No fear, 4=Extreme fear), please indicate your own puppy's recent tendency to display fearful behaviour in each of the following circumstances:

	0	1	2	3	4	Not Appl.
When approached directly by an unfamiliar adult while away from your home.	O	O	O	O	O	O
When approached directly by an unfamiliar child while away from your home.	O	O	O	O	O	O
In response to sudden or loud noises (e.g., vacuum cleaner, car backfire, road drills, objects being dropped, etc.).	O	O	O	O	O	O
When unfamiliar persons visit your home.	O	O	O	O	O	O
When an unfamiliar person tries to touch or pet the puppy.	O	O	O	O	O	O
In heavy traffic	O	O	O	O	O	O
In response to strange or unfamiliar objects on or near the pavement (e.g., plastic trash bags, leaves, litter, flags flapping...).	O	O	O	O	O	O
When examined/treated by a veterinarian.	O	O	O	O	O	O
During thunderstorms, firework displays, or similar events.	O	O	O	O	O	O
When approached directly by an unfamiliar dog of the same or larger size.	O	O	O	O	O	O
When approached directly by an unfamiliar dog of a smaller size.	O	O	O	O	O	O
When first exposed to unfamiliar situations (e.g., first car trip, first time in elevator, first visit to veterinarian, etc.)	O	O	O	O	O	O
In response to wind or wind-blown objects.	O	O	O	O	O	O
When manipulated by a household member (e.g.,, bathing, grooming, toweling feet, looking at ears...)	O	O	O	O	O	O
When stepped over by a member of the household.	O	O	O	O	O	O
When unfamiliar dogs visit your home.	O	O	O	O	O	O

When barked, growled, or lunged at by an unfamiliar dog.	O	O	O	O	O	O

SECTION 4: *Separation-related behaviour*

Some dogs show signs of anxiety or abnormal behaviour when left alone, even for relatively short periods of time. Thinking back over the recent past, how often has your puppy shown each of the following signs of separation-related behaviour when left, or about to be left, on its own (check appropriate boxes)?

	Never	Seldom	Sometimes	Usually	Always	Not Appl.
Shaking, shivering or trembling.	O	O	O	O	O	O
Excessive salivation.	O	O	O	O	O	O
Restlessness/agitation/pacing.	O	O	O	O	O	O
Whining.	O	O	O	O	O	O
Barking.	O	O	O	O	O	O
Howling.	O	O	O	O	O	O
Excessive chewing/scratching at doors, floor, windows, curtains, etc.	O	O	O	O	O	O
Loss of appetite.	O	O	O	O	O	O

SECTION 5: *Excitability*

Some dogs show relatively little reaction to sudden or potentially exciting events and disturbances in their environment, while others become highly excited at the slightest novelty. Signs of mild to moderate excitability include increased alertness, movement toward the source of novelty, and brief episodes of barking. Extreme excitability is characterised by a general tendency to over-react. The excitable dog barks or yelps hysterically at the slightest disturbance, rushes towards and around any source of excitement, and is difficult to calm down.

Calm: little or no special reaction	**Mild—Moderate excitability:** **0.....1.....2.....3.....4**	**Extremely excitable:** over-reacts, hard to calm down.

Using the following 5-point scales (0=Calm, 4=Extremely excitable), please indicate your own puppy's recent tendency to become excitable in each of the following circumstances:

	0	1	2	3	4	Not Appl.
When you or other members of the household come home after a brief absence.	O	O	O	O	O	O
When playing with you or other members of your household.	O	O	O	O	O	O
When doorbell rings.	O	O	O	O	O	O

Just before being taken on a trip or for a walk.	O	O	O	O	O	O
When visitors arrive at your home.	O	O	O	O	O	O

SECTION 6: *Attachment and Attention-seeking*

Most dogs are strongly attached to their people, and some demand a great deal of attention and affection from them. Thinking back over the recent past, how often has your puppy shown each of the following signs of attachment or attention seeking?

	Never	Seldom	Sometimes	Usually	Always	Not Appl.
Displays a strong attachment for one particular member of the household.	O	O	O	O	O	O
Tends to follow you (or other members of household) about the house, from room to room.	O	O	O	O	O	O
Tends to sit close to, or in contact with, you (or others) when you are sitting down.	O	O	O	O	O	O
Tends to nudge, nuzzle or paw you (or others) for attention when you are sitting down.	O	O	O	O	O	O
Becomes agitated (whines, jumps up, tries to intervene) when you (or others) show affection for another **person**.	O	O	O	O	O	O
Becomes agitated (whines, jumps up, tries to intervene) when you show affection for another dog or **animal**.	O	O	O	O	O	O

SECTION 7: *Miscellaneous*

Dogs display a wide range of miscellaneous behaviour in addition to those already covered by this questionnaire. Thinking back over the recent past, please indicate how often your puppy has shown any of the following behaviours:

	Never	Seldom	Sometimes	Usually	Always	Not Appl.
Chases or would chase cats given the opportunity.	O	O	O	O	O	O
Chases or would chase birds given the opportunity.	O	O	O	O	O	O
Chases or would chase squirrels, rabbits and other small animals given the opportunity.	O	O	O	O	O	O
Rolls in animal droppings or other ‘smelly’ substances.	O	O	O	O	O	O
Eats own or other animals’ droppings or feces.	O	O	O	O	O	O
Chews inappropriate objects.	O	O	O	O	O	O
Mounts’ objects, furniture, or persons.	O	O	O	O	O	O
Begs persistently for food when people are eating.	O	O	O	O	O	O

Steals food.	O	O	O	O	O	O
Nervous or frightened on stairs.	O	O	O	O	O	O
Pulls excessively hard when on the leash.	O	O	O	O	O	O
Urinates against objects/ furnishings in your home.	O	O	O	O	O	O
Urinates when approached, petted, handled or picked up.	O	O	O	O	O	O
Urinates when left alone at night, or during the daytime.	O	O	O	O	O	O
Defecates when left alone at night, or during the daytime.	O	O	O	O	O	O
Hyperactive, restless, has trouble settling down.	O	O	O	O	O	O
Often playful, boisterous.	O	O	O	O	O	O
Active, energetic, always on the go.	O	O	O	O	O	O
Stares intently at nothing visible.	O	O	O	O	O	O
Snaps at (invisible) flies.	O	O	O	O	O	O
Chases own tail/hind end.	O	O	O	O	O	O
Chases/follows shadows, light spots, etc.	O	O	O	O	O	O
Barks persistently when alarmed or excited.	O	O	O	O	O	O
Licks him/herself excessively.	O	O	O	O	O	O
Licks people or objects excessively.	O	O	O	O	O	O

If you have comments, please mention it in the text box below:

7.3. Puppy C-BARQ values for each breeder type

Table Z: puppy C-BARQ VALUES (± standard deviation) for each breeder type

(n=203; Min. score value=0; Max. score value=4)

	Small-scale breeders		Large-scale breeders		
	A	B	C	D	MEDIAN
Stranger-Directed Aggression	0.34 (0.63)	0.34 (0.32)	0.16 (0.28)	0.18 (0.64)	0.27
Owner-Directed Aggression	0.26 (0.50)	0.12 (0.16)	0.22 (0.43)	0.10 (0.22)	0.20
Dog-Directed Aggression	0.60 (0.94)	0.19 (0.26)	0.38 (0.60)	0.36 (0.93)	0.45
Stranger-Directed Fear	0.42 (0.80)	0.55 (0.86)	0.51 (1.08)	0.17 (0.66)	0.38
Nonsocial Fear	1.00 (0.81)	0.77 (0.55)	0.96 (0.96)	0.66 (0.72)	0.89
Dog-Directed Fear	0.86 (1.01)	0.30 (0.33)	0.60 (1.00)	0.59 (0.78)	0.70
Separation-Related Behavior	0.77 (0.66)	0.61 (0.67)	0.74 (0.62)	0.89 (0.72)	0.76
Attachment and Attention Seeking	2.02 (0.80)	2.08 (0.83)	1.81 (0.64)	2.09 (0.71)	2.00
Trainability	3.43 (0.76)	2.91 (0.96)	3.20 (0.73)	3.25 (0.53)	3.32
Chasing	1.19 (0.95)	1.21 (1.04)	1.03 (0.97)	0.69 (1.03)	1.07
Excitability	1.73 (0.87)	1.58 (0.88)	1.46 (0.83)	1.52 (0.89)	1.64
Touch Sensitivity	0.39 (0.69)	0.39 (0.59)	0.45 (0.89)	0.26 (0.58)	0.37
Energy Level	1.59 (0.75)	1.61 (0.56)	1.70 (0.91)	1.46 (0.71)	1.60
Dog Rivalry	0.33 (0.79)	0.25 (0.35)	0.50 (0.82)	0.10 (0.17)	0.31

A: occasional dog breeders: up to 9 dams on site

B: occupational dog breeders: From 10 to 50 dams on site

C: commercial dog breeders: More than 50 dams on site

D: dog merchants: Up to 50 dams on site and more than 350 puppies sold yearly

8. REFERENCES

Barnard, S., Siracusa, C., Reisner, I., Valsecchi, P., Serpell, J.A., 2012. Validity of model devices used to assess canine temperament in behavioral tests. Appl. Anim. Behav. Sci. 138, 79–87. https://doi.org/http://dx.doi.org/10.1016/j.applanim.2012.02.017

Beaudet, R., Chalifoux, A., Science, A.D.-A.A.B., 1994, undefined, Dallaire, A., 1994. Predictive value of activity level and behavioral evaluation on future dominance in puppies. Appl. Anim. Behav. Sci. 40, 273–284. https://doi.org/10.1016/0168-1591(94)90068-X

Blackwell, E.J., Twells, C., Seawright, A., Casey, R.A., 2008. The relationship between training methods and the occurrence of behavior problems, as reported by owners, in a population of domestic dogs. J. Vet. Behav. Clin. Appl. Res. 3, 207–217. https://doi.org/10.1016/j.jveb.2007.10.008

Clarke, A.S., Wittwer, D.J., Abbott, D.H., Schneider, M.L., 1994. Long-term effects of prenatal stress on HPA axis activity in juvenile rhesus monkeys. Dev. Psychobiol. 27, 257–269. https://doi.org/10.1002/dev.420270502

Coppinger, R., Coppinger, L., 2001. Dogs :a startling new understanding of canine origin, behavior, and evolution. Scribner, New York.

Dahlöf, L.-G., Hård, E., Larsson, K., 1978. Influence of maternal stress on the development of the fetal genital system. Physiol. Behav. 20, 193–195.

Dalkey, N., Helmer, O., 1963. An Experimental Application of the DELPHI Method to the Use of Experts. Manage. Sci. 9, 458–467. https://doi.org/10.1287/mnsc.9.3.458

Dalla Villa, P., Barnard, S., Di Nardo, A., Iannetti, L., Vulpiani, M.P., Trentini, R., Serpell, J.A., Siracusa, C., Chiave Aggressività, P., 2017. Validation of the Socially Acceptable Behaviour (SAB) test in a Central-Italy pet dog population. Vet. Ital. 53, 61–70. https://doi.org/10.12834/VetIt.321.1283.3

Day, M.J., Horzinek, M.C., Schultz, R.D., Squires, R.A., 2016. WSAVA Guidelines for the vaccination of dogs and cats [WWW Document]. J. Small Anim. Pract. https://doi.org/10.1111/jsap.2_12431

Dendoncker, P.-A., De Keuster, T., Diederich, C., Dewulf, J., Moons, C., 2019. On the origin of puppies: Breeding and selling procedures relevant for canine behavioural development. Vet. Rec. https://doi.org/http://dx.doi.org/10.1136/vr.104979

Dendoncker, P.-A., de Rooster, H., Abma, E., Wydooghe, E., Dewulf, J., 2018. Biosecurity measures for dog merchants and canine breeding kennels, in: Dewulf, J., Van Immerseel,

F. (Eds.), Biosecurity in Animal Production and Veterinary Medicine: From Principles to Practice. Acco, Leuven / Den Haag, pp. 433–452.

Dendoncker, P.-A.J.A.F., Lensen, C.M.M., Moons, C.P.H., De Keuster, T., Dewulf, J., Diederich, C., 2015. Adaptation of an existing behavior assessment tool for use in puppies. J. Vet. Behav. Clin. Appl. Res. 10, 445. https://doi.org/10.1016/j.jveb.2015.07.025

Diederich, C., Giffroy, J.-M., 2006. Behavioural testing in dogs: A review of methodology in search for standardisation. Appl. Anim. Behav. Sci. 97, 51–72. https://doi.org/http://dx.doi.org/10.1016/j.applanim.2005.11.018

Dodman, N.H., Brown, D.C., Serpell, J.A., 2018. Associations between owner personality and psychological status and the prevalence of canine behavior problems. PLoS One 13, e0192846. https://doi.org/10.1371/journal.pone.0192846

Döring, D., Roscher, A., Scheipl, F., Küchenhoff, H., Erhard, M.H., 2009. Fear-related behaviour of dogs in veterinary practice. Vet. J. 182, 38–43. https://doi.org/10.1016/J.TVJL.2008.05.006

Duffy, D.L., Kruger, K.A., Serpell, J.A., 2014. Evaluation of a behavioral assessment tool for dogs relinquished to shelters. Prev. Vet. Med. 117, 601–609. https://doi.org/http://dx.doi.org/10.1016/j.prevetmed.2014.10.003

Duffy, D.L., Serpell, J.A., 2012a. Predictive validity of a method for evaluating temperament in young guide and service dogs. Appl. Anim. Behav. Sci. 138, 99–109. https://doi.org/10.1016/j.applanim.2012.02.011

Duffy, D.L., Serpell, J.A., 2012b. Predictive validity of a method for evaluating temperament in young guide and service dogs. Appl. Anim. Behav. Sci. 138, 99–109. https://doi.org/http://dx.doi.org/10.1016/j.applanim.2012.02.011

Dupont, S., Butaye, P., Claerebout, E., Theuns, S., Duchateau, L., Van de Maele, I., Daminet, S., 2013. Enteropathogens in pups from pet shops and breeding facilities. J. Small Anim. Pract. 54, 475–480. https://doi.org/10.1111/jsap.12119

Fatjo, J., Amat, M., Mariotti, V.M., de la Torre, J.L.R., Manteca, X., 2007. Analysis of 1040 cases of canine aggression in a referral practice in Spain. J. Vet. Behav. Clin. Appl. Res. 2, 158–165. https://doi.org/10.1016/j.jveb.2007.07.008

Fox, M.W., 1972. Understanding your dog. Coward, McCann & Geoghegan.

Fox, M.W., 1971. Integrative development of brain and behavior in the dog. University of Chicago Press, Chicago.

Fox, M.W., Stelzner, D., 1966. Behavioural effects of differential early experience in the dog. Anim. Behav. 14, 273–281. https://doi.org/10.1016/S0003-3472(66)80083-0

Foyer, P., Bjällerhag, N., Wilsson, E., Jensen, P., 2014. Behaviour and experiences of dogs during the first year of life predict the outcome in a later temperament test. Appl. Anim. Behav. Sci. 155, 93–100. https://doi.org/10.1016/j.applanim.2014.03.006

Frank, D., 2014. Recognizing Behavioral Signs of Pain and Disease: A Guide for Practitioners. Vet. Clin. North Am. Small Anim. Pract. 44, 507–524. https://doi.org/10.1016/J.CVSM.2014.01.002

Fratkin, J.L., Sinn, D.L., Patall, E.A., Gosling, S.D., 2013. Personality Consistency in Dogs: A Meta-Analysis. PLoS One 8, e54907. https://doi.org/10.1371/journal.pone.0054907

Gazzano, A., Mariti, C., Notari, L., Sighieri, C., McBride, E.A., 2008. Effects of early gentling and early environment on emotional development of puppies. Appl. Anim. Behav. Sci. 110, 294–304. https://doi.org/10.1016/j.applanim.2007.05.007

Godbout, M., Palestrini, C., Beauchamp, G., Frank, D., 2007. Puppy behavior at the veterinary clinic: A pilot study. J. Vet. Behav. Clin. Appl. Res. 2, 126–135. https://doi.org/10.1016/j.jveb.2007.06.002

Goddard, M.E., Beilharz, R.G., 1982. Genetic and environmental factors affecting the suitability of dogs as Guide Dogs for the Blind. Theor. Appl. Genet. 62, 97–102. https://doi.org/10.1007/BF00293339

Gómez, J.-C., 2005. Species comparative studies and cognitive development. Trends Cogn. Sci. 9, 118–125. https://doi.org/10.1016/J.TICS.2005.01.004

Goodman, C.M., 1987. The Delphi technique: a critique. J. Adv. Nurs. 12, 729–734. https://doi.org/10.1111/j.1365-2648.1987.tb01376.x

Hird, D., Ruble, R., Reagor, Sg.D.D. et al 1992. pdfFARE/Hir. et al 1992. pd., 1992. Morbidity and mortality in pups from pet stores and private sources: 968 cases (1987-1988). J. Am. Vet. Med. Assoc. 201, 471–474.

Hoskins, J.D., Partington, B.P., 2001. Chapter 1 - Physical Examination and Diagnostic Imaging Procedures, in: Hoskins, J.D. (Ed.), Veterinary Pediatrics (Third Edition). W.B. Saunders, Philadelphia, pp. 1–21. https://doi.org/http://dx.doi.org/10.1016/B978-0-7216-7665-4.50005-X

Hsu, Y., Serpell, J.A., 2003. Development and validation of a questionnaire for measuring behavior and temperament traits in pet dogs. J. Am. Vet. Med. Assoc. 223, 1293–1300.

Hsu, Y., Sun, L., 2010. Factors associated with aggressive responses in pet dogs. Appl. Anim. Behav. Sci. 123, 108–123. https://doi.org/10.1016/J.APPLANIM.2010.01.013

Immelmann, K., Suomi, S.J., 1981. Sensitive phases in development, Behavioral Development. Cambridge University Press.

Jones, A.C., Gosling, S.D., 2005. Temperament and personality in dogs (Canis familiaris): A review and evaluation of past research. Appl. Anim. Behav. Sci. 95, 1–53. https://doi.org/10.1016/J.APPLANIM.2005.04.008

Konok, V., Dóka, A., Miklósi, Á., 2011. The behavior of the domestic dog (Canis familiaris) during separation from and reunion with the owner: A questionnaire and an experimental study. Appl. Anim. Behav. Sci. 135, 300–308. https://doi.org/10.1016/j.applanim.2011.10.011

Koolhaas, J.., Korte, S.., De Boer, S.., Van Der Vegt, B.., Van Reenen, C.., Hopster, H., De Jong, I.., Ruis, M.A.., Blokhuis, H.., 1999. Coping styles in animals: current status in behavior and stress-physiology. Neurosci. Biobehav. Rev. 23, 925–935. https://doi.org/10.1016/S0149-7634(99)00026-3

Kurdek, L.A., 2008. Pet dogs as attachment figures. J. Soc. Pers. Relat. 25, 247–266. https://doi.org/10.1177/0265407507087958

Landis, J.R., Koch, G.G., 1977. The Measurement of Observer Agreement for Categorical Data. Biometrics 33, 159. https://doi.org/10.2307/2529310

Lee, P.R., Brady, D.L., Shapiro, R.A., Dorsa, D.M., Koenig, J.I., 2007. Prenatal stress generates deficits in rat social behavior: Reversal by oxytocin. Brain Res. 1156, 152–167. https://doi.org/10.1016/j.brainres.2007.04.042

Lensen, C.M.M., Lebocq, M., Moons, C.P.H., Diederich, C., 2013a. Evaluation of veterinarians' and dog owners' ability to interpret communication signals in dogs, in: Proceedings of the 2nd International Symposium of Veterinary Ethology. SEEVAD, Lyon; FR, p. 13.

Lensen, C.M.M., Moons, C.P.H., Diederich, C., 2014. Puppy behavior during a clinical exam as assessed by veterinarians – Preliminary results. J. Vet. Behav. 9, e1. https://doi.org/10.1016/j.jveb.2014.09.006

Lensen, C.M.M., Moons, C.P.H., Diederich, C., 2013b. Training website for the behavioral assessment of pet dogs [WWW Document]. URL http://pupwelfare.be/vet/training/ (accessed 3.7.19).

Ley, J.M., Bennett, P.C., Coleman, G.J., 2009. A refinement and validation of the Monash Canine Personality Questionnaire (MCPQ). Appl. Anim. Behav. Sci. 116, 220–227. https://doi.org/10.1016/j.applanim.2008.09.009

Lund, E.M., Armstrong, P.J., Kirk, C.A., Kolar, L.M., Klausnor, J., 1999. Health status and population characteristics of dogs and cats examined at private veterinary practices in the United States. J. Am. Vet. Med. Assoc. 214, 1336–1341.

Mariti, C., Raspanti, E., Zilocchi, M., Carlone, B., Gazzano, A., 2015. The assessment of dog welfare in the waiting room of a veterinary clinic. Anim. Welf. 24, 299–305. https://doi.org/10.7120/09627286.24.3.299

McMillan, F.D., 2017. Behavioral and psychological outcomes for dogs sold as puppies through pet stores and/or born in commercial breeding establishments: Current knowledge and putative causes. J. Vet. Behav. Clin. Appl. Res. 19, 14–26. https://doi.org/10.1016/j.jveb.2017.01.001

McMillan, F.D., Serpell, J.A., Duffy, D.L., Masaoud, E., Dohoo, I.R., 2013. Differences in behavioral characteristics between dogs obtained as puppies from pet stores and those obtained from noncommercial breeders. J. Am. Vet. Med. Assoc. 242, 1359–1363. https://doi.org/10.2460/javma.242.10.1359

Mirkó, E., Kubinyi, E., Gácsi, M., Miklósi, Á., 2012. Preliminary analysis of an adjective-based dog personality questionnaire developed to measure some aspects of personality in the domestic dog (Canis familiaris). Appl. Anim. Behav. Sci. 138, 88–98. https://doi.org/10.1016/j.applanim.2012.02.016

Morrow, M., Ottobre, J., Ottobre, A., Neville, P., St-Pierre, N., Dreschel, N., Pate, J.L., 2015. Breed-dependent differences in the onset of fear-related avoidance behavior in puppies. J. Vet. Behav. 10, 286–294. https://doi.org/10.1016/J.JVEB.2015.03.002

Nagasawa, M., Kanbayashi, S., Mogi, K., Serpell, J.A., Kikusui, T., 2016. Comparison of behavioral characteristics of dogs in the United States and Japan. J. Vet. Med. Sci. 78, 231–8. https://doi.org/10.1292/jvms.15-0253

Nagasawa, M., Mogi, K., Kikusui, T., 2012. Continued Distress among Abandoned Dogs in Fukushima. Sci. Rep. 2, 724. https://doi.org/10.1038/srep00724

Nagasawa, M., Tsujimura, A., Tateishi, K., Mogi, K., Ohta, M., Serpell, J. a, Kikusui, T., 2011. Assessment of the factorial structures of the C-BARQ in Japan. J. Vet. Med. Sci. 73, 869–875. https://doi.org/10.1292/jvms.10-0208

Nagle, T., 2006. Topics in pediatric dermatology. Vet. Clin. North Am. Small Anim. Pract. 36, 557–572.

Overall, K.L., 2013. Manual of clinical behavioral medicine for dogs and cats. Elsevier Mosby Publishing, St. Louis.

Overall, K.L., Hamilton, S.P., Chang, M.L., 2006. Understanding the genetic basis of canine anxiety: phenotyping dogs for behavioral, neurochemical, and genetic assessment. J. Vet. Behav. 1, 124–141. https://doi.org/10.1016/J.JVEB.2006.09.004

Patronek, G.J., Glickman, L.T., Beck, A.M., McCabe, G.P., Ecker, C., 1996. Risk factors for relinquishment of dogs to an animal shelter. J. Am. Vet. Med. Assoc. 209, 572–81.

Piaget, J., 1977. The development of thought : equilibration of cognitive structures. Viking Press, New York.

Pierantoni, L., Albertini, M., Pirrone, F., 2011. Prevalence of owner-reported behaviours in dogs separated from the litter at two different ages. Vet. Rec. 169. https://doi.org/10.1136/vr.d4967

Pirrone, F., Pierantoni, L., Mazzola, S.M., Vigo, D., Albertini, M., 2015. Owner and animal factors predict the incidence of, and owner reaction toward, problematic behaviors in companion dogs. J. Vet. Behav. Clin. Appl. Res. https://doi.org/10.1016/j.jveb.2015.03.004

Pirrone, F., Pierantoni, L., Pastorino, G.Q., Albertini, M., 2016. Owner-reported aggressive behavior towards familiar people may be a more prominent occurrence in pet shop-traded dogs. J. Vet. Behav. Clin. Appl. Res. 11, 13–17. https://doi.org/10.1016/j.jveb.2015.11.007

Riemer, S., Müller, C., Virányi, Z., Huber, L., Range, F., 2014. The Predictive Value of Early Behavioural Assessments in Pet Dogs – A Longitudinal Study from Neonates to Adults. PLoS One 9, e101237. https://doi.org/10.1371/journal.pone.0101237

Root Kustritz, M. V, 2011. Chapter 4 - History and Physical Examination of the Weanling and Adolescent, in: Kutzler, M.E.P.A. (Ed.), Small Animal Pediatrics. W.B. Saunders, Saint Louis, pp. 28–33. https://doi.org/http://dx.doi.org/10.1016/B978-1-4160-4889-3.00004-8

Scarlett, J.M., Saidla, J.E., Pollock, R. V, 1994. Source of acquisition as a risk factor for disease and death in pups. J. Am. Vet. Med. Assoc. 204, 1906–13.

Scott, J., 1958. Critical periods in the development of social behavior in puppies. Psychosom. Med. 20, 42–54.

Scott, J.P., Fuller, J.L., 1965. Genetics and the Social Behavior of the Dog. University of Chicago Press, Chicago.

Shaw, J.R., Adams, C.L., Bonnett, B.N., Larson, S., Roter, D.L., 2008. Veterinarian-client-patient communication during wellness appointments versus appointments related to a health problem in companion animal practice. J. Am. Vet. Med. Assoc. 233, 1576–1586. https://doi.org/10.2460/javma.233.10.1576

Strandberg, E., Jacobsson, J., Saetre, P., 2005. Direct genetic, maternal and litter effects on behaviour in German shepherd dogs in Sweden. Livest. Prod. Sci. 93, 33–42. https://doi.org/10.1016/j.livprodsci.2004.11.004

Svartberg, K., 2002. Shyness–boldness predicts performance in working dogs. Appl. Anim. Behav. Sci. 79, 157–174. https://doi.org/10.1016/S0168-1591(02)00120-X

Takahashi, L.K., Turner, J.G., Kalin, N.H., 1992. Prenatal stress alters brain catecholaminergic activity and potentiates stress-induced behavior in adult rats. Brain Res. 574, 131–137. https://doi.org/10.1016/0006-8993(92)90809-N

Tamimi, N., Jamshidi, S., Serpell, J.A., Mousavi, S., Ghasempourabadi, Z., 2015. Assessment of the C-BARQ for evaluating dog behavior in Iran. J. Vet. Behav. Clin. Appl. Res. 10, 36–40. https://doi.org/10.1016/j.jveb.2014.09.064

Temesi, A., Turcsán, B., Miklósi, Á., 2014. Measuring fear in dogs by questionnaires: An exploratory study toward a standardized inventory. Appl. Anim. Behav. Sci. 161, 121–130. https://doi.org/10.1016/J.APPLANIM.2014.09.009

Tiira, K., Lohi, H., 2015. Early Life Experiences and Exercise Associate with Canine Anxieties. PLoS One 10, e0141907. https://doi.org/10.1371/journal.pone.0141907

Tiira, K., Sulkama, S., Lohi, H., 2016. Prevalence, comorbidity, and behavioral variation in canine anxiety. J. Vet. Behav. 16, 36–44. https://doi.org/10.1016/J.JVEB.2016.06.008

Tuchscherer, M., Kanitz, E., Otten, W., Tuchscherer, A., 2002. Effects of prenatal stress on cellular and humoral immune responses in neonatal pigs. Vet. Immunol. Immunopathol. 86, 195–203. https://doi.org/10.1016/S0165-2427(02)00035-1

van den Berg, L., Schilder, M.B.H., Vries, H. de, Leegwater, P.A.J., van Oost, B.A., 2006. Phenotyping of Aggressive Behavior in Golden Retriever Dogs with a Questionnaire. Behav. Genet. 36, 882–902. https://doi.org/10.1007/s10519-006-9089-0

van den Berg, S.M., Heuven, H.C.M., van den Berg, L., Duffy, D.L., Serpell, J.A., 2010. Evaluation of the C-BARQ as a measure of stranger-directed aggression in three common dog breeds. Appl. Anim. Behav. Sci. 124, 136–141. https://doi.org/10.1016/j.applanim.2010.02.005

Volk, J.O., Felsted, K.E., Thomas, J.G., Siren, C.W., 2011. Executive summary of the Bayer veterinary care usage study. J. Am. Vet. Med. Assoc. 238, 1275–1282. https://doi.org/10.2460/javma.238.10.1275

Weiss, E., Greenberg, G., 1997. Service dog selection tests: Effectiveness for dogs from animal shelters. Appl. Anim. Behav. Sci. 53, 297–308. https://doi.org/10.1016/S0168-1591(96)01176-8

Wilsson, E., 1984. The social interaction between mother and offspring during weaning in German shepherd dogs: individual differences between mothers 1. Wilsson E. 1984; The social interaction between mother and offspring during weaning in German shepherd dogs: individual d. Appl. Anim. Behav. Sci. 13, 101–112. https://doi.org/https://doi.org/10.1016/0168-1591(84)90056-X

Wilsson, E., Sundgren, P.-E., 1998a. Effects of weight, litter size and parity of mother on the behaviour of the puppy and the adult dog. Appl. Anim. Behav. Sci. 56, 245–254. https://doi.org/https://doi.org/10.1016/S0168-1591(97)00094-4

Wilsson, E., Sundgren, P.-E., 1998b. Behaviour test for eight-week old puppies—heritabilities of tested behaviour traits and its correspondence to later behaviour. Appl. Anim. Behav. Sci. 58, 151–162. https://doi.org/http://dx.doi.org/10.1016/S0168-1591(97)00093-2

Wright, J.C., 1983. The effects of differential rearing on exploratory behavior in puppies. Appl. Anim. Ethol. 10, 27–34. https://doi.org/10.1016/0304-3762(83)90109-8

CHAPTER 7:
GENERAL DISCUSSION

The overarching purpose of this research was to describe current dog breeding practices and to assess the behaviour and health of puppies sold in Belgium. More specifically, we investigated the presence of a link between the source of acquisition and aspects important to the behavioural development of puppies on the one hand and to health management and biosecurity of the breeding facility on the other hand. Three studies were conducted in order to assess the breeders' practices relevant for the aspects of interest and to assess the behaviour and the health of puppies before and after homing. This dissertation documents how the various types of dog breeders in Belgium operate and to which extent differences in outcomes are imputable to their organisation. Our research has also permitted the identification of areas where improvements are desirable and achievable.

Dog breeders vary greatly in the size of their operations and in the organisation of their breeding facility. This resulted in differences in organisation, in socialisation opportunities for puppies, in the risk and impact of disease, etc. Instead of generalising all dog breeders, in this research a tailored approach was retained by classifying dog breeder into different categories. The combination of an overview of current practices (chapters 3 and 4) together with measured outcomes on behaviour and health of puppies (chapters 5 and 6) gave insight into similarities and differences between different breeder categories and provided indications about the impact of the environment, shaped by the breeder.

This broad and multidisciplinary approach tends to differ from most studies. Most investigate the environment shaped by the dog breeder focussing either on diseases and associated risk factors (Grellet et al., 2012a, 2014; Mircean et al., 2012; Scarlett et al., 1994) or on the behavioural development in early life (Gazzano et al., 2008; Harvey et al., 2016; Korbelik et al., 2011; Taylor and Mills, 2007; Tiira and Lohi, 2015). However, as dog breeders operate with a convergence of both aspects, our research included both of these aspects. This enabled us to not only identify possible improvements for dog breeding practices, but also to provide non-conflicting (Stepita et al., 2013) recommendations for pet dog breeders and contracting veterinarians towards improving welfare for breeding stock and limiting behavioural impairments and health issues of puppies.

1. AREAS OF IMPROVEMENT IN THE CLASSIFICATION OF DOG BREEDER TYPES.

To date, dog breeders are categorised based on Belgian legislation, resulting in 7 categories (see introduction for a detailed description). Categorising dog breeders permits a risk-based approach and allows the institutions to legislate and regulate accordingly. However, the legislative categories revealed to have limitations. During our study, we discovered several issues with the existing classification for breeders. First, the thresholds applied by the legislative classification to define the size of a facility are very close. More specifically, differences between categories with regard to the number of dams are minimal, resulting in similar types of breeders being classified over multiple categories, which was expected. The similarity revealed to extend beyond the size of the facility. We observed similarities in organisation, management and practices. As a result, a relatively low variation was observed between legislative categories with regard to the number of dams (depicted in figure 7.1) and habits influencing the behavioural development of puppies (e.g., daily access outside, raising puppies in a household (chapter 3) and for commonly applied biosecurity measures (e.g.,quarantine of dogs, hygienic measures) (chapter 4).

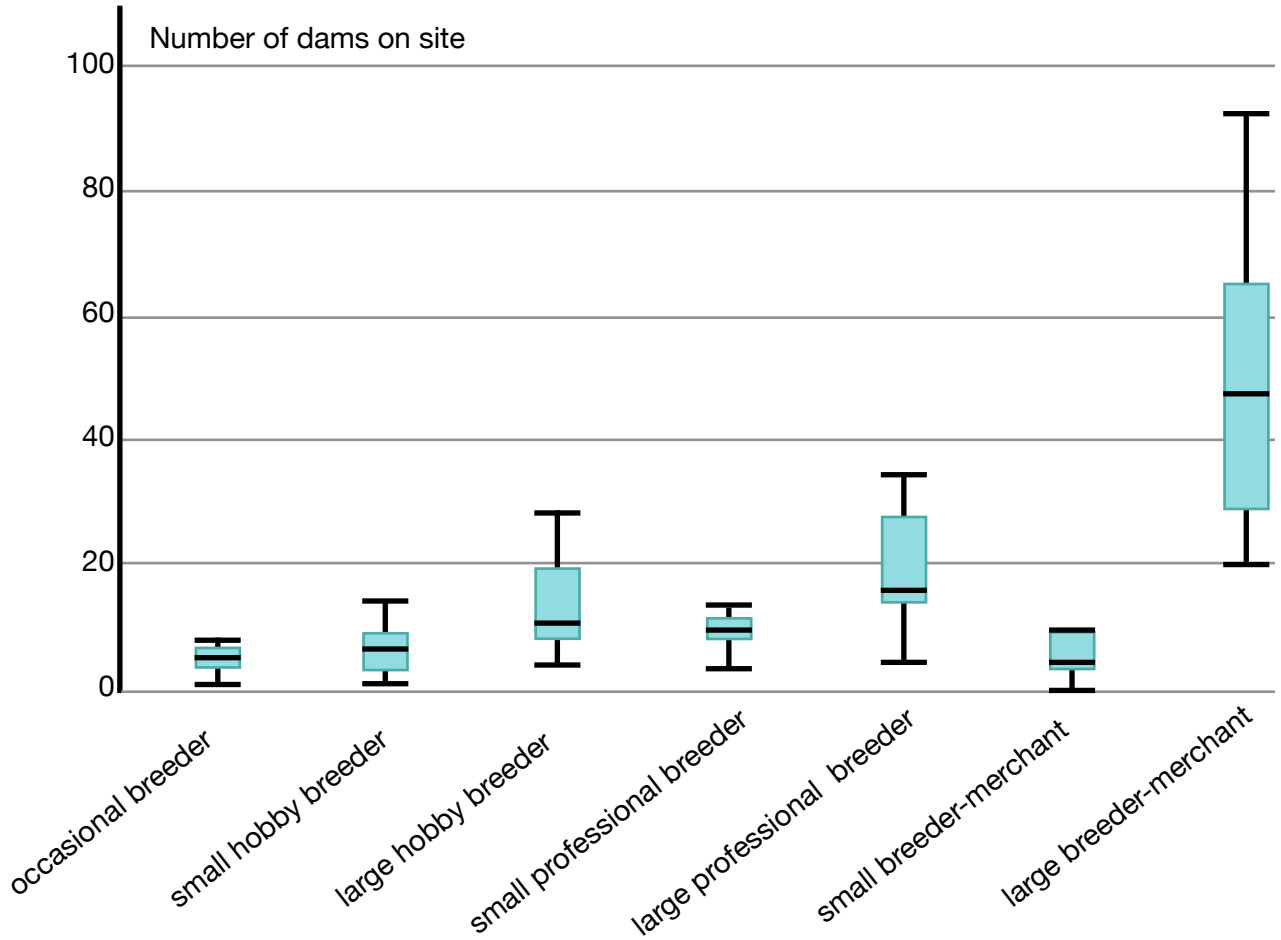

Figure 7.1: Graphical representation of the number of dams on site for each legislative category

Second, the legal classification revealed not to be respected by all breeders. Eighteen percent of breeders visited had more dams than allowed by their registration, and 12% of the breeders produced more litters yearly than allowed by their registration number (chapter 4). Additionally, some dog breeders combined two or more registration numbers for their breeding facility. This discrepancy caused even less variation between breeders than what was initially expected. Therefore, for the purpose of our research, the legislative categories were of little value. Consequently, we developed a different classification, by factor segmentation of breeders by means of the observed number of dams on-site and the declared yearly number of puppies sold. A segment was defined as a group of breeders that is organised and perform practices relevant to hygiene, health management, and behavioural development of puppies in a like manner.

The number of dams on site (and, indirectly, the number of sires) was preserved from the original classification because it is easily applicable and was observed to be a valuable parameter to define the scale of a dog breeding facility. The thresholds were adapted to increase the inter-categorical variability. The yearly number of litters produced was evaluated, since this is the main determinant of the legislative categories and this number defines the number of production cycles throughout the year. However, since Belgian breeders are not allowed to produce more than two litters per year with one dam, both are somewhat redundant. Additionally, the annual production does not take into account the occupancy rate of the breeding facility since litter size varies greatly (Mickelsen et al., 1993; Okkens et al., 1993), nor does it reflect the span of trade activities. Therefore, our alternative classification retained the number of puppies reported to be sold per year. A detailed description of these categories can be found in chapter 3. With the new classification we defined thresholds based on the observed animals on site and used larger ranges between categorical thresholds. This resulted in a decrease in heterogeneity within breeder categories for general management (e.g., staff, children involved) and practices (e.g., hygienic measures, outdoor access), and an increase of between-breeder category heterogeneity, rendering it more useful. A graphical representation of this increased interclass heterogeneity is depicted in figure 7.2. The modified categories were used throughout all chapters in this dissertation.

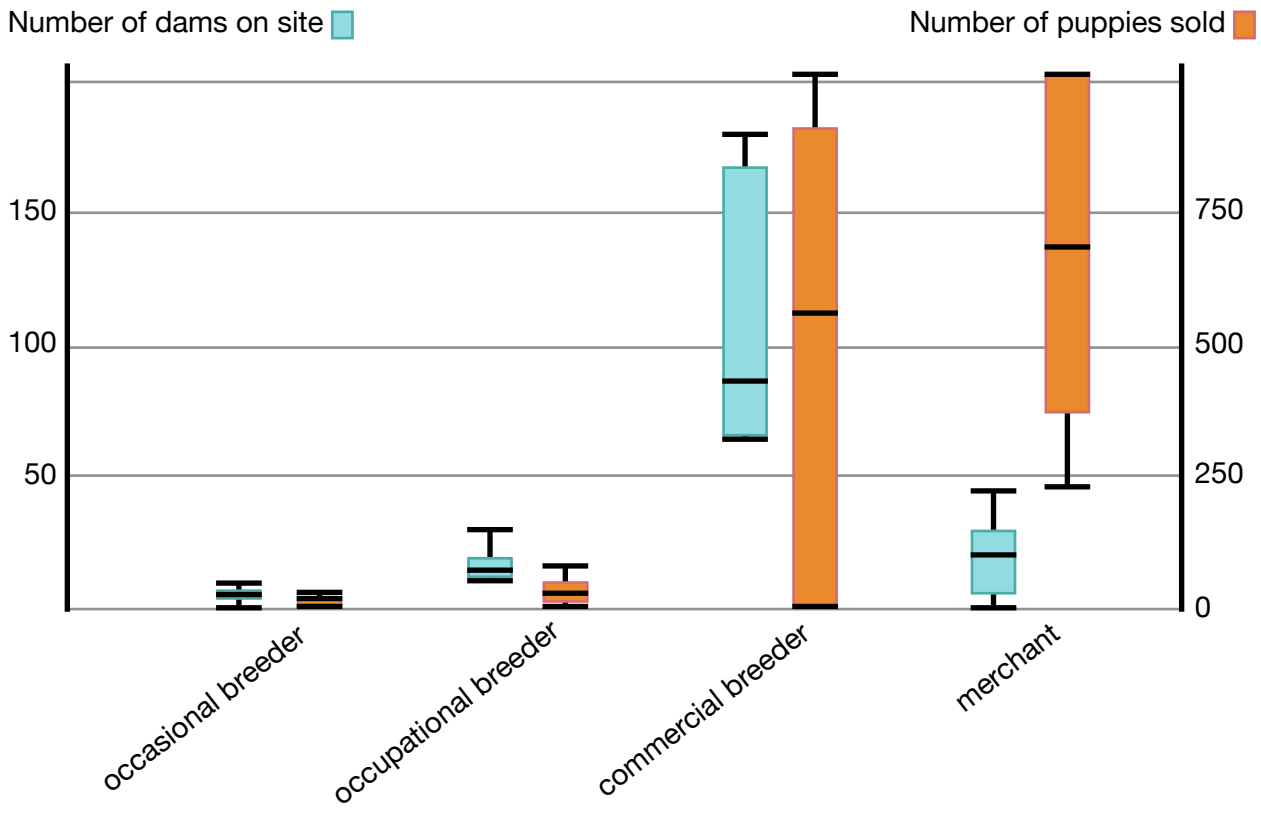

Figure 7.2: Graphical representation of the number of dams on site for each alternative category

2. AREAS OF IMPROVEMENT IN THE TRACEABILITY OF DOGS WITH A FOREIGN ORIGIN

During this research, 29% of puppies had passports issued outside of Belgium, and 19% were imported commercially. In the veterinary screening of health, we found no significant difference in health issues for puppies originating from abroad. Higher occurrence of symptoms in these puppies revealed to be an age effect. Age fraud is often anecdotally reported by veterinarians. Not surprisingly, almost half of puppies with a passport issued in Czech Republic looked younger than the registered age. This suspicion, however, was not confirmed by the details provided about teething (chapter 6). A more thorough study of teething and other age-parameters would be advisable to elucidate this discrepancy. Meanwhile, registration of all the important life stages of a puppy, from birth to homing and including veterinary check-ups, in a European database would facilitate governments to regulate and could possibly impede such fraud.

Dog merchants involved in intracommunity trade anecdotally described the procedure as follows: puppies are acquired from whitelisted foreign merchants (mainly in Slovakia and Czech

Republic), which are subjected to comply to Belgian legislation and compliance is assured by local authorities. The whitelisted merchants source puppies from different sized local breeders. Upon departure from the country of origin, puppies are registered in the trade control and expert system (TRACES) and are delivered by registered transporters on a fixed day of the week. The breeds and number of acquired dogs depend on the offer and demand. One dog merchant stated that adherence to this arrangement is unsure because of the multiple players involved. A lack of transparency was anecdotally reported from participating veterinarians. A more detailed traceability is advisable, because, in analogy to production animals directed at the food chain, it could decrease the uncertainty and speculations surrounding international trade. For instance, the microchip number could be linked to a freely accessible European database reporting all movements of the puppy, including original breeder, consulted veterinarians, involved merchants and transporters.

Interestingly, in chapter 6 we also collected data of imported puppies from neighbouring countries (7 from the Netherlands, 5 from France, out of 219) although these countries are almost absent in commercial import statistics (number of puppies registered in TRACES and imported into Flanders in 2016: 61 from France, 16 from the Netherlands and none from Germany). This means that the dogs imported from these countries were likely brought in via parallel import or may be subject to the illegal circuit. While parallel import of dogs is a fundamental right (treaty of Schengen), our findings confirm that it is not a marginal phenomenon. Because of the unregistered and uncontrolled nature of non-commercial transport of dogs, it possibly facilitates fraud and opens the door for illegal import, not only diminishing animal welfare but also increasing the risks of importing diseases. Illegal import of dogs from foreign countries is considered the main risk factor for rabies in Belgium (Sciensano, 2019). While Intra-Community movements is of limited risk, isolated cases of rabies involving illegally smuggled infected dogs do occur. The risks of legal import is even more limited, at least if a proper immunization is achieved by respecting the schedules and minimal ages. A registration of non-commercial transport of dogs would be of added value. This would provide better statistics to identify the span of the problem, subsequently this would allow improved targeting of suspicious movements by authorities and more efficient control.

3. AREAS OF IMPROVEMENT IN CURRENT DOG BREEDING PRACTICES

From the results of our research and the available literature, we have identified areas of possible improvement with regard to early socialisation and environmental learning of puppies, health management, and biosecurity of the breeding facility. Although selection of breeding stock and mating strategies is potentially important for behavior and health, these breeding practices were out of scope of this research. Below, we focus on the implemented practices that tend to conflict with the current recommendations, practices that were applied at a relatively low rate, or practices that were poorly performed.

3.1. Dog breeding practices with a focus on behavioural development of puppies

3.1.1. Limiting situations perceived as stressful and threatening during early-life

Although stress is a fundamental ability of dogs, frequently or chronically elevated stress hormones may have detrimental consequences for welfare, health and behavioural development (see introduction for a detailed description). Unappropriated housing, such as a monotonous environment that limits the species-specific behaviour, causes an increase in frustration in adult dogs and, subsequently, stress (Protopopova, 2016). Nonsocial (Pullen et al., 2010) and social enrichment (Hubrecht, 1993; Valsecchi et al., 2007) resulting in diverse stimuli is a strategy to limit these adverse effects (Wells, 2004). In puppies, inappropriate housing may not provide a strong basis for proper socialisation (De Meester et al., 2005). Providing diverse novel stimuli is a common method for rearing puppies (Seksel et al., 1999). In our study, we observed a more enriched environment and a greater variety of stimuli at small-scale dog breeders (chapter 3).

The absence of stimuli is not the only cause of stress. A major source of frustration and stress in animals is the lack of control over their environment (Mason et al., 2007). Obvious stressful stimuli, such as electrical shock (Solomon et al., 1953) or excessive noise (Beerda et al., 1997), have long been acknowledged. However, this also includes not being able to avoid contact with unfamiliar humans, such as visitors (Merola et al., 2012; Vas et al., 2005). Fearful reactions can even occur towards familiar humans, such as a caretaker (Lefebvre et al., 2009). In our study, owners that bought a puppy from an occasional breeder more often described their puppy as

fearful towards strangers compared to puppies from dog merchants (chapter 3). In parallel, puppies from commercial breeders performed more exploration of their pen (chapter 5) and were least often scored as fearful in a veterinary practice (chapter 6).

Contrary to common belief, it is safe to conclude there is no such thing as one common recipe to provide a social pet dog. Individual variation in stress susceptibility (Ebner and Singewald, 2017) and coping with stressful situations (Koolhaas et al., 2010) has been recently acknowledged. Environmental learning by puppies is mainly the result of habituation (Appleby, 1993). However, this only occurs when a weak stimulus is presented repeatedly (Rankin et al., 2009). Providing a stimulus that an individual finds frightening will, contrary to the purpose, lead to sensitisation (De Keuster et al., 2015). Additionally, fearful reactions of the dam may reinforce the reaction of the puppies, because dams will act as a secure base (Previde et al., 2009).

Human-dog interactions might lead to conflicts because the behavioural responses of dogs to human-dog interaction may be misinterpreted and wrongly assessed. The communication of dogs is based on body postures and facial expressions and eventually includes physical contact. On the contrary, humans use verbal and tactile signals to communicate with dogs (Kuhne et al., 2012). Gaining knowledge in the behavioural repertoire of dogs will provide insight in the dog's emotional state and its perception of the situation (Mariti et al., 2012).

It is advisable for all breeders to provide appropriated housing, fulfilling physical needs, but also providing diverse stimuli (olfactory, auditory, visual) in the form of nonsocial and social enrichment to dams and puppies. Meanwhile, it is important to provide a dog with the choice to move away from a stimulus it finds frightening. Last, all persons involved in dog activities should gain knowledge of the canine behavioural repertoire and proactively adapt the situation and provided stimuli suited to the individual dog.

3.1.2. Children involved in daily care of breeding stock and puppies

Introducing children at an early age to work-related practices is a common phenomenon in small- and medium-sized family owned businesses (Birley et al., 1999). This is even more so the case in dog breeding facilities, where it is a popular belief that all puppies must interact with children as much as possible during the canine socialisation period ("Socialising puppies and dogs | Blue Cross,"). Half of the breeders (except merchants) involved under-aged children (<

16 years) in their daily practices. Involving children may be useful for the socialisation of children and of dogs, and could result in a possible decrease of inappropriate responses by the dogs towards children later in life (Arai et al., 2011). From a breeder's point of view, the benefits of this practice can also represent a cost reduction, by reducing outsource to staff. However, there are a few caveats to this practice. First, dogs represent a risk for children since bite incidents are common and children are more likely to experience dog bites than adults (De Keuster et al., 2006; Schalamon et al., 2006). The responsible adult must supervise and should provide proper bite-prevention education for the children (Meints and de Keuster, 2009). This is even more so the case with pregnant dams who are prone to react more aggressively (Kustritz, 2005). Second, children involved in the daily care must be educated properly and should be able to recognise stress signals while interacting with dogs (Mariti et al., 2012). Third, the general hygiene and biosecurity measures in the breeding facility, such as wearing gloves and boot covers or the use of dedicated clothes and equipment per compartment, must always be respected. Adapting biosecurity measures to the children's age and situation is a necessity (Bradley, 2007). Lastly, the children should always be reminded of hygienic measures, so as to reduce their risk for contracting a zoonosis (Robertson et al., 2000).

3.1.3. Raising puppies in a household-like environment

The presence of the household-like environment is inversely proportional with the scale of the breeding facility. The majority of occasional (88%) and occupational breeders (66%) declared to raise puppies at least partially in a household-like environment, while this practice is less common in commercial breeders (33%) and merchants (40%) (chapter 4). Raising puppies in a household-like environment is generally accepted as the best way to prepare dogs to future life as a companion dog. Providing the puppy with an environment that is similar to the environment after homing is very useful. It provides puppies with diverse nonsocial and social stimuli and better habituates them to the situations they most likely will encounter later in life. Additionally, most private houses are well insulated and equipped with temperature control (STATBEL, 2018) which results in more comfortable housing for young puppies.

The greater fear perceived in puppies from occasional breeders after homing may indicate that the raising of puppies in a household-like environment may also entail certain attention points. The higher amount and intensity of stimuli in a household-like environment can, for some

puppies, be perceived as an excess. Loud TV-noises, grooming or even the presence of teenagers have been suggested to elicit fearful reactions. Therefore, a matching of the environment before homing to the environment likely to be experienced in adulthood, must be performed by assessing individual reactions of each puppy and by taking into account fearful personalities.

Additionally, the complexity of a furnished private home can be a challenge for proper cleaning and disinfection (Kagan et al., 2002). In essence, porous materials and fabrics have small interstices that can be resistant to biocides, making them more difficult to properly clean and disinfect. Frequent use of porous surfaces was observed most in the breeders that provided a household-like environment (chapter 4). Lastly, some human (electrical) appliances form a potential hazard for puppies who discover the world by snapping and biting.

An improvement for breeders that raise puppies in a household-like environment (essentially small-scale and occasional breeders) would be to limit the area of the household to which puppies have access. First, this will allow the breeder to have more control over the interactions of the puppy and limit the nonsocial and social stimuli perceived as stressful. Second, this will allow a better implementation of biosecurity principles (e.g., limitation of movements of inhabitants, restriction to cleanable surfaces) and a better prevention of hazards (avoiding power outlets and cables, small objects, etc.). Increasing the scale of the breeding facility in a household-like environment makes it difficult to implement these measures and maintain control. Instead of trying to create a household-like environment in large-scale breeding facilities, improvements for large-scale breeders would rather be to apply the biosecurity principles during the nonsocial and social enrichment, to respect hygienic measures when interacting with the puppies, and to organise the nursery in such a way that it provides a controlled amount of stimuli (Loveridge, 1998).

3.1.4. Daily outdoor access for puppies

Providing outdoor access has been described as a way to increase the activity level and mobility of adult dogs (Spangenberg et al., 2006), to provide environmental enrichment and to decrease the frequency of unwanted behaviours (Khoshnegah et al., 2011) generally associated with confinement of adult dogs in restricted indoor spaces (Hetts et al., 1992; Hurt and Croney,

2016; McMillan et al., 2011). The effect is expected to be of less importance for puppies in breeding facilities (Cannas et al., 2010), considering the short period at the breeder (chapter 3). Outdoors will provide additional nonsocial and social stimuli which may be interesting for the environmental learning and socialisation (e.g., discriminating between surfaces appropriate for urination and defecation and surfaces appropriate for other activities, more olfactory stimuli, larger exercise space, etc.).

A downside of providing outdoor access is that this is a less controllable environment, often containing uncontrolled stimuli (e.g., noises). A second downside is that common outdoor surfaces (e.g., grass, dirt) are difficult to clean and disinfect and may hasten the reinfection of environmentally stable pathogens. This downside, however, is easily corrected by paving the outdoor surfaces with concrete or pavement, which are easier to clean and disinfect (Dewulf and Van Immerseel, 2018).

Organising a private outdoor area and increasing the awareness of the risks associated with (public) outdoor areas will limit health and behavioural threats, while increasing the welfare. All breeders should organise their private outdoor area to comply with the general biosecurity measures of the facility (e.g., divide exercise areas into different zones, one for each compartment, avoid crossing of compartments, etc.). Breeders anecdotally reported to consider the weather conditions before letting a puppy outdoors. However, a sheltered exercise area can be provided when the weather is not appropriate for young puppies (Loveridge, 1998). If a private outdoor area is not available, specific considerations should be made before allowing puppies into public areas. First, to confront a young puppy with multiple new situations will increase the risk of stimuli at an intensity above its stress threshold. Second, a higher venue of conspecifics will increase the soil load and thus the risk of contracting pathogens (Martínez-Moreno et al., 2007; Overgaauw and van Knapen, 2013; Smith et al., 2015). Lastly, a seasonal effect can be expected for common canine pathogens (Gordon and Angrick, 1986; Rika-Heke et al., 2015). If no private outdoor is available, breeders should avoid public areas with high attendance in their neighbourhood and adapt their habits in order to avoid the highest pathogen load (Westgarth et al., 2010). An improvement for all breeders would be to provide more control over every outdoor situation.

3.1.5. Providing toys to puppies

In this research, all breeders were observed to provide toys to their puppies (chapter 3 and 6). Toys provide diverse, nonsocial stimuli to puppies, support exploratory behaviour, and favour the environmental learning (Pullen et al., 2010). Toys not only enrich the environment but also permit puppies to explore different textures, noises, shapes, etc. Additionally, chewing toys supports the development of masticatory muscles and helps the transition from suckling to chewing (Iinuma et al., 1991). However, research on the value of toys for puppies revealed that not all toys elicit the same quality of interaction (Hubrecht, 1993; Pullen et al., 2012, 2010). Inappropriate toys (or the ones that elicit an inappropriate behaviour) may create possible health hazards for puppies (Hayes, 2009).

Toys must be suited (size, weight) and safe (strength, hardness, shape, materials) to the age and size of the puppies. A crucial part of providing toys consists of observation: individual preferences exist (Hubrecht, 1993) and some toys may elicit aggression between dogs. General principles of biosecurity apply for toys as well: toys can be dedicated per compartment or cleaned when rotating between compartments. It is advisable to use only toys that are able to withstand wear and can be cleaned and disinfected, or that will be disposed of after use. It is preferable to include toys in the cleaning routine. Suspended toys should be considered because they increase the mental and physical stimulation, while facilitating cleaning procedures.

3.2. Dog breeding practices with a focus on health management

3.2.1. Vaccination of breeding stock and puppies

Vaccination is defined by the World Health Organisation (WHO) as the active immunisation of an individual against a particular disease by administration of a biological preparation (“WHO | Vaccines,” 2017). International scientific recommendations identify core vaccines, that all dogs, regardless of the circumstances, should receive. They protect dogs from severe, life-threatening pathogens that have a global distribution, including CDV, CAV, and CPV2 (Day et al., 2016). Additionally, vaccination does not significantly contribute to ill-health in companion animals (Day, 2006). Available data suggest that the overall prevalence of true adverse reactions to

vaccination is exceedingly low: retrospective studies in the US revealed that 0.4% of dogs presented vaccine-associated adverse events (Yao et al., 2015; Moore et al., 2005).

Vaccination also prevents and controls infectious diseases at the level of the breeding facility. The vaccination of breeding stock will protect not only sires and dams, but also puppies through passive immunisation. The passive immunisation will depend on several factors, ranging from the personal immuno-competence of the puppy (Toman et al., 2002), to antibody-levels of the dam (Coyne et al., 2001). Maternally derived antibodies (MDA) are mainly obtained by ingestion of colostrum (Pollock and Carmichael, 1982) and marginally by transplacental transfer (Stoffel et al., 2000). MDA will interfere with the active immunisation one wants to achieve with vaccination (Day, 2007). As a result, immunisation of young puppies is not straightforward (Decaro et al., 2005).

In our study, vaccination of breeding stock was declared to be applied at high rate by merchants, commercial, occupational and occasional breeders (100%, 100%, 93% and 85% respectively) (chapter 4). Further improvements can be made by following the actual scientific guidelines when vaccinating breeding stock with core vaccines (Day et al., 2016). Breeding stock acquired as a puppy should be vaccinated at 16 weeks of age and receive a booster injection following the primo-vaccination 12 months later (Schultz et al., 2010). Iteration should not be more frequently than every three years, because the duration of immunity is many years and may, in fact, last for the lifetime of the pet (Schultz et al., 2010)

Although we collected that the importance of vaccination of puppies is widely acknowledged and primo-vaccination of puppies is highly applied, ranging 89% to 97% depending on breeder category (chapter 6), improvements are possible and advisable. Considering the high vaccination rate of breeding stock (chapter 4), it is to be expected that most dams will have high titres against CPV. They will transfer enough MDA to their offspring (Hernández-Blanco and Catala-López, 2015). Primo-vaccination of puppies however, will only provide the aimed immunisation once MDA are sufficiently decreased (De Cramer et al., 2011; Winters, 1981), generally somewhere between 8 and 16 weeks. Core vaccines administered shortly before homing (i.e., at 8 weeks) should, therefore, be repeated at 12 and 16 weeks (Day et al., 2016). A vaccination of puppies younger than 8 weeks is facultative when a passive immunisation by the dam was provided.

An additional recommendation towards dog breeders (and veterinarians performing puppy consultations) is to inform dog owners of this current scientific consensus. These recommendations can diverge from the information that can be found in the package insert of the vaccine (i.e., vaccination at 6, 8 and 12 weeks only). Although the vaccination schedule that appears in the package insert has been reviewed and approved by the licensing authorities, the practical use of the vaccine in the field can deviate from this official schedule. Furthermore, new scientific data can modify the vaccination approach, but the respective changes in the regulations can take several years before coming into force (Thiry and Horzinek, 2007).

The vaccination scheme can be further improved by the contracting veterinarian based on regional prevalence (e.g., leptospirosis) in outbreak situations or in case of suspicion of disease. The adjustments can be guided by additional diagnostics, for instance it is possible to check the number of antibodies in the breeding animals by performing a titration (Twark and Dodds, 2000). Measuring a dog's titre of antibodies specific to a particular pathogen gives insight into the active immunisation state of the dog. When performed after vaccination, this practice ensures a proper seroconversion. Titration permits the contracting veterinarian to customise the vaccination protocol according to actual immunisation state instead of applying an approximate scheme. Sometimes, titration can reduce the number of vaccinations, for instance, when it appears that a dog still has a sufficient amount of antibodies at the time of a booster vaccination.

Vaccination has no particular goal with regard to the behavioural development and the effect of vaccination on welfare and behavioural development was not researched. However, low-stress handling has to be retained, especially when performing a vaccination of puppies. Because performing the titration (i.e., blood collecting by means of venipuncture) can increase stress (Hennessy et al., 1998), it is preferable to either establish a positive association with veterinarians before blood collection, or to refrain from titration until adult age.

3.2.2. Endoparasite control (deworming) of breeding stock and puppies

Endoparasite control of breeding stock and puppies, especially deworming, has two major aims: to kill helminth infestations that are potentially harmful for dogs and/or humans and to reduce the amount of helminth eggs shed into the environment. Hereby, deworming reduces helminth infection of unweaned puppies, limits zoonoses, and increases the health and welfare of the breeding dogs. Low-stress handling or creating a positive association with performing the deworming will limit the impact on the emotional health of the puppy. An additional benefit is an optimised food conversion of breeding dogs and growth of puppies.

All commercial breeders and merchants (100%) and the vast majority of occupational (95%) and occasional breeders (85%) declared in our survey to apply routine deworming of breeding stock (chapter 4). All merchants, commercial and occupational breeders (100%) and the vast majority of occasional breeders (95%) also declared to perform regular endoparasite control of their puppies (chapter 4). However, the anecdotal data that we collected does not indicate proper knowledge of the current scientific guidelines, which is to apply a broad-spectrum anthelmintic treatment in adult dogs every season, and to treat all puppies at 2, 4 ,6 and 8 weeks, subsequently monthly to six months of age (ESCCAP, 2010). An improvement would be to perform endoparasite treatment following these guidelines.

Additionally, in our study, a moderate number of owners (58%) declared to their veterinarian during the first puppy consultation that their puppy was dewormed at least once by the breeder (regardless of breeder category). Surprisingly, only few owners (23%, regardless of the breeder category) had a detailed knowledge about the deworming status of their puppy (chapter 6). An improvement would be that all breeders (and veterinarians performing puppy consultation) take the time to provide clear information (e.g., handouts) to dog owners of previously performed endoparasite control and to provide future treatment recommendation based on the current scientific consensus.

Last, in our study a combined formulation of metronidazole and spyramicin was found to be routinely administered to puppies suspected of, but not diagnosed with, giardiasis (chapter 4). The contracting veterinarian should always be contacted in case of an outbreak or suspicion of disease so as to perform additional diagnostics and, if necessary, apply therapeutic strategies.

3.2.3. Use of antimicrobials in pregnant dams and puppies

The periparturient period is undoubtedly the period with the highest risk of disease transmission and antimicrobial treatments (i.e., amoxicillin-clavulanic acid or cephalosporins) are readily available and applied during this period (Milani et al., 2012; Münnich, 2008). However, the use of these protocols may, contrarily to their purpose, give rise to unwanted or even dangerous circumstances such as the development of drug resistance. Antimicrobial resistance in dogs has already been shown for *Enterococcus* strains and *Staphylococcus* strains (Bramble et al., 2011; De Graef et al., 2004; Rota et al., 2013), which can be the result of misuse in breeding kennels (Rota et al., 2011). Even more, the utility of such practice with regard to morbidity and mortality is questionable (Milani et al., 2012). In our study, a considerable part (ranging 13-24%) of the questioned breeders declared to administer antimicrobials systematically to dams around parturition (chapter 4). To what extent antimicrobials could play a role in neurotransmission and behavioural development of dogs is currently unknown. An improvement is to abandon systematic administration of antimicrobials. Any kind of antimicrobial treatment should always be based upon correct diagnosis of the pathogen and ideally an antibiogram, to prevent antimicrobial resistance.

3.3. Dog breeding practices with a focus on biosecurity

3.3.1. Quarantine of externally acquired dogs

Our results indicated that breeders regularly acquire dogs (i.e., puppies) from external sources (annual renewal average is 11% of the breeding stock) to maintain or increase their breeding stock (chapter 4). Because it is a mandatory measure for certain breeder categories (*RD 27/04/2007*), we found that quarantine was implemented by all commercial breeders (100%), moderately by merchants (71%) and rarely by occupational and occasional breeders (25 and 3% respectively) (chapter 4). We collected anecdotally, however, that the method of implementation of quarantine is questionable. First, it is advisable that during quarantine, all animals suspected as carrier of pathogens are segregated in a dedicated compartment, until these animals have been observed for a minimum time frame or tested to be pathogen free. It is also mandatory that, during this period, the caretaker complies to the hygienic measures in order not to break the compartmentalisation. This means washing hands and changing

footwear or using disposable gloves and boot-covers before entering and after leaving the quarantine, and reserving apparel and equipment dedicated to this quarantine. The use of a specific colour scheme for the quarantine is a practical way to make these perceptible to all.

In our study we also observed anecdotally that the implementation of quarantine could result in complete isolation. For dogs, restricting the ability to engage in species-specific behaviours (Taylor and Mills, 2007), for instance by spatial and social isolation, is a stressful situation (Hennessy et al., 1997). Effects of longer duration of sheltered dogs (which provide similar segregation to quarantine) are contradictory (reviewed by Protopopova, 2016). However, prolonged stressful situations may introduce behavioural impairments of breeding stock (McMillan et al., 2011) which possibly transfers to offspring (Cushing and Kramer, 2005). Improvements in the housing conditions during quarantine are advisable. The quarantine room must therefore not only comply to the physical needs (e.g., minimal space, control of temperature and ventilation, natural light schedule, etc.) but also provide social and nonsocial enrichment to fulfil the emotional needs of dogs of different ages (Coppinger and Zuccotti, 1999; Hubrecht, 1993).

Social enrichment without breaking the compartmentalisation can be achieved, not only by quarantining pairs or groups of dogs during the quarantine period, but also by providing regular positive interactions with the caretakers. During social interactions, limitations of the quarantine can be respected by endorsing strict hygienic measures such as a hygiene lock, proper hand hygiene, dedicated apparel and equipment. In larger facilities, a caretaker dedicated to the areas at risk (e.g., quarantine, sick bay, etc.) could be considered. Non-social enrichment is possible by providing toys that are not only safe and stimulating, but that also are able to withstand wear and are routinely cleaned and disinfected. Alternatively, these toys could be dedicated to the quarantine pen and then thrown away. Creating more stimulating premises, for instance by including a platform, can also be considered, as long as cleaning and disinfection possibilities are addressed in the added complexity.

3.3.2. Regulation of visitors in the maternity ward.

The maternity ward is the place where the dam is isolated and where puppies are housed until weaning. Providing a maternity ward accommodates the natural behaviour of the expectant

dam, who prepares for the birth by withdrawing from her usual social interactions. She becomes more sedentary and prepares a "nest" site (Kustritz, 2005). Visitors can be granted access for different reasons (e.g., veterinarian, future owner, maintenance technician, etc.). Occasional breeders grant access more easily (43%) while this practice is more limited by occupational breeders, commercial and merchants (24%, 14% and 13% respectively) (chapter 3). Based on previous research, visitors could be perceived as a social enrichment for the dam, and could counter the stress associated with confinement (Beerda et al., 1999; Mariti et al., 2014). However, the interaction provided by strangers will most likely be a less positive interaction than interaction with familiar humans, and can even, by some dams, be perceived as a threat (Kuhne et al., 2014; Vas et al., 2005). Therefore, the temperament of the dam must always be taken into account when deciding to allow visitors access to the maternity. In mammals, repeated exposure to unpredictable stressful situations may not only suppress the immunological activity of the dam (Sapolsky et al., 2000) but may also influence the maternal behaviour (Bosch and Neumann, 2012; Neumann et al., 2005) and will negatively impact the immune response (Tuchscherer et al., 2002) and the early behavioural development of the offspring (Austin et al., 2005; Braastad, 1998; Czerwinski et al., 2016).

Additionally, puppies in the maternity are the most at risk to contract a disease because of their immature immunity and their higher susceptibility for common pathogens (Day, 2007; Münnich and Lübke-Becker, 2004). Therefore, limiting and regulating visitors in the maternity ward will not only limit unpredictable stressful situations but will also decrease the risk of entrance of pathogens in the maternity. Regulating the access to visitors is common practice in merchants, commercial and occupational breeders (100%, 100%, and 89% respectively) and is fairly common in occasional breeders (78%) (chapter 4). The risks associated with an unregulated access of visitors seems to be least acknowledged in occasional breeders, especially if the visitors are familiar persons or relatives. Puppies from this category were found by their owners to be more fearful towards strangers compared to the puppies from merchants, and also showed more fear-related behaviour in the veterinary practice compared to puppies from commercial breeders (chapter 6).

An improvement would be to limit visitors to only those that are absolutely necessary (e.g., primary caretaker, veterinarian in case of disease, future owner) and by regulating and

supervising all access to the maternity ward. Because the primary caretaker will mostly function as a secure base (Gácsi et al., 2013; Horn et al., 2013), there are indications that positive interactions with the dam and early gentling of puppies should be procured mainly by the caretakers. With the omnipresence of cameras and social media, there is no need to provide access to the maternity for future owners. Also professional visitors (e.g., veterinarians) should be limited to the necessary. Regulation would permit the breeder to ensure that all visitors are properly educated when interacting with the dogs in the maternity ward (Firnkes et al., 2017). Essentially, regulation of visitors is advisable because it will support the behavioural development of puppies and limit the risks of disease.

3.3.3. Pest control

Pests are considered to be all animals that are detrimental to a human activity or that form a nuisance to humans, companion and production animals. In dog breeding activities, rodents, birds and arthropods (all insects and arachnids, but i.e., ectoparasites) can be considered as pests, mainly because of their negative impact on the hygiene and health management of the facility. During large infestations, rodents will damage premises and equipment and consume or spoil food, increasing all-round costs. Also, rodents (Jansen et al., 2005), insects (Shaw et al., 2009) and ticks (Solano-Gallego et al., 2016) can harbour and spread canine pathogens (Day, 2011). Finally, most pests will impact the fitness and welfare of stocked animals. In our study, rodent control was frequently applied by commercial breeders (86%), moderately by merchants (50%) and seldom by occupational and occasional breeders (24% and 17% respectively). On the contrary, control of arthropods (mainly by ectoparasitic treatment) was performed moderately by occasional and occupational breeders (43% and 49% respectively) and seldom by commercial breeders and merchants (29%) (chapter 4). In our screening of health after homing, 13% of all puppies were infested by ectoparasites (mainly *Ctenocephalides felis*), regardless of breeder category (chapter 6).

Pests will generally only be recognised when large infestations are present. The caretakers should check regularly for the presence of pests by placing specific traps (rodents) and grooming the dogs (arthropods). Because of the variety of pests, it is necessary to implement the right measures depending on the pest identified (e.g., specific rodenticide at key locations

and limiting propitious material for nesting in order to counter a large rodent infestation). The staff performing pest control should limit the stress for the dogs, ideally making all handling a positive experience. Specifically when applying topical formulations (i.e., ectoparasitic treatment) on puppies, a low-stress handling is advisable.

Good pest control starts with appropriate detection and identification of the pest. It supports the applied biosecurity measures (e.g., damaged surfaces are harder to clean) and counters the negative impact on physical (i.e., introduction of pathogens) and emotional (e.g., itch, sleep disruption because of nocturnal activities) health of all housed dogs.

3.3.4. Hygienic measures at the maternity ward and the nursery

General hygienic measures are aimed at limiting the spread of pathogens between the dam or her offspring and dogs from other compartments (e.g., weaned puppies, adult dogs). Hygienic measures will also reduce the entrance of pathogens from outside (e.g., veterinary practice, other kennels) and additionally limit the zoonotic risks. Pregnant dams and young puppies are less immunocompetent than dogs of other ages and are more at risk for contracting a disease (Evermann and Wills, 2011; Ronsse et al., 2005). Later, at age of weaning, puppies are most at risk for enteric pathogens (Grellet et al., 2014, 2012b). These pathogens originate from adult dogs, environment or vectors, and are mainly transmitted via faecal-oral route (Patel and Heldens, 2009; Sokolow et al., 2005).

Hygienic measures consist of routinely washing hands, wearing appropriate footwear, the use of dedicated apparel, etc. Handling of neonate puppies is common practice for paediatric care (Lawler, 2008) and additionally ensures improved behavioural development (Gazzano et al., 2008). Since tactile contact between caretaker or visitors and dogs is mainly performed with hands, hand hygiene is of utmost importance. Footwear hygiene and dedicated clothes will limit the spread of pathogens through fomites and decrease the entrance of pathogens into the maternity. Not only the staff, but also the visitors must be made aware of this. Routine application of hygienic measures in the maternity ward was often absent in small-scale breeders (adoption rate: 21%) and only moderately present in large-scale breeders (60%), with 33% even limiting hygienic measures to hand hygiene (washing hands or wearing disposable gloves) only. The adoption rate of routine hygienic measures in the nursery was even lower in

large-scale breeders (33%) and almost non-existent in small-scale breeders (8%) (chapter 4). This is alarming considering the widespread of enteric viruses (i.e., CPV and CCoV). The presence of enteric pathogens could be a direct result of the poor implementation of hygienic measures. Because the absence of hygienic measures will increase the risk of transmission of pathogens (Hoelzer and Parrish, 2010), all breeders would stand to benefit from implementing a routine application of hygienic measures.

3.3.5. Cleaning procedures at the maternity ward and the nursery

Cleaning is the process of removing organic material and the majority of the microbial population. Because maternal pathogens (e.g., *E. coli, B. bronchiseptica, Streptococcus* spp., *Staphylococcus* spp.) can cause neonatal morbidity and puppy mortality (Münnich, 2008; Nielen et al., 1998). Puppies at weaning age are also at risk, especially for enteric pathogens. Common in breeding facilities are: *T. canis, Cystoisospora* sp., *Giardia* sp., *Hammondia*-like protozoa, CPV, CCoV, and *E. coli* (Claerebout et al., 2009; Dupont et al., 2013).

During our study, daily removal of faeces was a practice widely applied in all breeder categories (on average once or more daily) in maternity ward and nursery. A thorough wet cleaning procedure was declared to be performed twice a week on average, regardless of breeder category (chapter 4). Forty-eight breeders declared to perform a wet cleaning once a week and six of them declared to perform it even less often. The widespread of enteric viruses could also be a result of incorrect cleaning procedures, or insufficient cleaning application, although this was not investigated.

Cleaning will effectively reduce the environmental load of most pathogens (Dewulf and Van Immerseel, 2018) and therefore limit neonatal diseases caused and faecal-oral transmission. Additionally, choice of detergent should always be based on the surface-type that is to be cleaned (Dewulf and Van Immerseel, 2018), and the maternity ward or nursery should be empty during the soaking of surfaces until completely dry. This will not only minimise puppies' exposure to high humidity (Xong et al., 2017) but will also limit the persistence (Olson et al., 1999) and dissemination (Exner et al., 2004) of pathogens in moisture. Therefore, all breeders stand to benefit from reconsidering their current cleaning strategy and implementing cleaning procedures recommended specifically for dog facilities (Gilman, 2004).

Cleaning of the maternity ward and nursery has no particular goal when it comes to behavioural development; however, concerns can arise regarding stressful or negative interactions. Entering the pen multiple times a day to remove faeces implies an interaction with the dam and her puppies. Regular interactions with the caretaker can be regarded as social enrichment (Lefebvre et al., 2009; Menor-Campos et al., 2011), precluding negative interaction. For instance, the use of high pressure equipment can be perceived as stressful (Castelhano-Carlos and Baumans, 2009; Coppola et al., 2006), and dam and puppies should not be nearby. If the interactions are positive and well-controlled, they can increase the welfare of the animals in the maternity ward, as this will serve as social enrichment and environmental learning. Additionally, it will provide a habituation to diverse environmental stimuli and to humans performing actions such as cleaning. In essence, a puppy must have the choice to move away from a stimulus it finds frightening, or it should not be present during the stimulus.

3.3.6. Disinfection of the maternity ward and the nursery

Disinfection is the process of decreasing the number of (or ideally destroying all) pathogens that remain after cleaning and has been proven to minimise diseases caused by pathogens. Because pregnant dams shed more pathogens and puppies in the maternity ward are most at risk (Münnich and Lübke-Becker, 2004; Schäfer-Somi et al., 2003), particular attention to the disinfection of the maternity ward and the nursery is required.

The success of disinfection will first and foremost depend on the precedent cleaning procedure (Terpstra et al., 2007). The organisation and surfaces of the maternity, including the whelping box, should be chosen on account of being easily cleanable and should not interact with the disinfectant (Böhm, 1998; L.J. Kagan, A.E. Aiello, 2002). The choice of disinfectant should be made with consideration for the targeted pathogens and the surface-types in the maternity (Eterpi et al., 2009; Fiechter et al., 2012). A proper application of the disinfectant, such as contact time, is necessary to ensure that the aimed pathogen reduction will be achieved in a considerable manner (Dewulf and Van Immerseel, 2018).

In our study, lower rates of disinfection were reported by occasional and occupational breeders (20% and 31% respectively) compared to merchants (88%), with commercial breeders in between (57%). These results indicate that there is room for improvement. Additionally, the

whelping box was frequently made of porous materials in all breeder categories (ranging from 33% to 71% depending on breeder category) which is more difficult to clean and disinfect properly (chapter 4). Fortunately, the scale at which occasional breeders operate results in production cycles that may be too far apart for most pathogens to remain virulent in the whelping box. However, the broad use of antimicrobials in pregnant dams around parturition (14% of all breeders) and in puppies (10% of all breeders) reported in chapter 4, may suggest the presence of recurrent infections (Milani et al., 2012). All breeders in all categories stand to benefit from gaining knowledge about the basic principles of disinfection. Because exposure to disinfectants can be harmful, it is advised to disinfect only when the room is empty and to let the puppies (and if present; the dam) back in only after all surfaces have completely dried.

Disinfection of the maternity ward and nursery has no particular goal with regard to the behavioural development and the effect on canine behaviour was not studied. However, the application might solicit reactions from the dam and her puppies. A thorough cleaning followed by disinfection can disrupt the olfactory landmarks and thus create a stressful situation (Taylor and Mills, 2007).

4. LIMITATIONS OF THIS RESEARCH

This research project pioneered the investigation of breeder type at the convergence of behavioral development and biosecurity. Considering the provided funding, existing literature, available assessment tools, required manpower and planned time, we focussed on developing the required tools and performing exploratory data collection and analysis. The methodological limitations were reported in the discussion of every research chapter. Recommendations towards researchers on how to address these limitations have been described in section 5.3. During this research, causation has not been investigated and associations reported in this dissertation should be interpreted and communicated accordingly.

5. RECOMMENDATIONS FOR EDUCATION, REGULATION, AND FUTURE RESEARCH

5.1. Education of dog breeders, contracting veterinarians and dog owners

More education on the principles of facility health management and biosecurity should be directed towards dog breeders and contracting veterinarians. Additionally, the actual scientific consensus surrounding vaccination and deworming should be easily available to contracting veterinarians. This would permit them to optimally guide dog breeders and ultimately recommend good vaccination and deworming practices to dog owners. Training of the contracting veterinarians could be realised by means of providing specific continuing education. In extension; the curriculum of small animal veterinarians could also include education on the guidance and medicine of dog facilities such as breeding kennels and shelters. In parallel, it is advisable to provide better education on the behavioural development of dogs and how to recognise stress signals in dogs for the dog breeders and future owners. Ideally, only persons that possess sufficient knowledge should be allowed to breed or sell dogs; for instance, by having to pass an exam to obtain a license or accreditation.

5.2. Recommendations towards the regulation of dog breeding facilities

As demonstrated in this research; the actual legislative classification of dog breeders is complicated, moderately applied in the field and mostly unknown by dog owners. It is advisable to reduce the current classification to fewer categories, separated by more distinct thresholds, and incorporate these categories into all bills regulating dog breeders. The classification of dog breeders should refrain from using suggestive nomenclature such as *hobby* or *commercial*, and the breeder category should be communicated clearly and compulsory towards potential buyers (the name and description instead of a registration number).

In order to limit the illegal circuit; all breeders categories should be registered. An improved traceability could be achieved by registering foreign origin and all intermediates, also for dogs that are the subject of parallel import.

5.3. Future research

During this research, we developed or modified several tools. The development and the modifications were necessary because at the onset of this project, no tools were available to achieve aimed purposes. However, more research is needed to test these tools in similar settings within different populations. For instance, a validation procedure of the behavioural questionnaires could be considered by matching the outcomes of the behavioral assessment by the veterinarian and the owner-directed questionnaire with a thorough consultation performed by a board-certified veterinary specialist in canine behaviour.

We retained a methodology that focussed on exploratory data collection and analysis. This approach permitted to describe husbandry practices at the convergence of behavioral development of puppies and biosecurity measures of the breeding facility and observe differences in behaviour and health. A more conclusive comparison of dog behaviour with regard to the origin could be achieved in an experimental setup. Additionally, the assessment of puppies and their functionality as a companion dog, would include repeated behavioural observations of the puppy and, later in life, of the adult dog. The application of biosecurity measures by dog breeders should ideally be measured by systematic observation instead of breeder declaration, and preferably by measuring several components of health and hygiene (e.g., incidence of disease, use of antimicrobials, bacterial load on surfaces). Ideally, these studies would be not limited to Belgium but be performed at European level in various populations.

During this research, we identified various gaps in current knowledge. The importance of coping styles in dogs and the influence of early personality and the development of undesirable behaviours such as stranger-directed aggression and fear remains to be elucidates. Additionally, more research is desirable about early-environmental factors of a dog's personality. We encountered several setbacks that mainly resulted in moderate sample sizes, therefore limiting the impact of our results. Replication of some of our studies could include modifications in methodology to increase the sample size. For instance, the behavioural assessment and screening of health could be reiterated by sampling puppies during identification (i.e., chipping) and registration instead of during the first puppy consultation.

6. REFERENCES

Appleby, D., 1993. Socialization and habituation, in: The Behavior of Dogs and Cats. London.

Arai, S., Ohtani, N., Ohta, M., 2011. Importance of Bringing Dogs in Contact with Children during Their Socialization Period for Better Behavior. J. Vet. Med. Sci. 73, 747–752. https://doi.org/10.1292/jvms.10-0445

Austin, M.P., Leader, L.R., Reilly, N., 2005. Prenatal stress, the hypothalamic-pituitary-adrenal axis, and fetal and infant neurobehaviour. Early Hum. Dev. 81, 917–926. https://doi.org/10.1016/j.earlhumdev.2005.07.005

Beerda, B., Schilder, M.B.., Bernadina, W., Van Hooff, J.A.R.A.., De Vries, H.W., Mol, J.A., 1999. Chronic Stress in Dogs Subjected to Social and Spatial Restriction. II. Hormonal and Immunological Responses. Physiol. Behav. 66, 243–254. https://doi.org/10.1016/S0031-9384(98)00290-X

Beerda, B., Schilder, M.B.H., van Hooff, J.A.R.A.M., de Vries, H.W., 1997. Manifestations of chronic and acute stress in dogs. Appl. Anim. Behav. Sci. 52, 307–319. https://doi.org/10.1016/S0168-1591(96)01131-8

Birley, S., Ng, D., Godfrey, A., 1999. The family and the business. Long Range Plann. 32, 598–608. https://doi.org/10.1016/S0024-6301(99)00076-X

Böhm, R., 1998. Disinfection and hygiene in the veterinary field and disinfection of animal houses and transport vehicles. Int. Biodeterior. Biodegradation 41, 217–224. https://doi.org/10.1016/S0964-8305(98)00030-4

Bosch, O.J., Neumann, I.D., 2012. Both oxytocin and vasopressin are mediators of maternal care and aggression in rodents: From central release to sites of action. Horm. Behav. 61, 293–303. https://doi.org/10.1016/J.YHBEH.2011.11.002

Braastad, B.O., 1998. Effects of prenatal stress on behaviour of offspring of laboratory and farmed mammals. Appl. Anim. Behav. Sci. 61, 159–180. https://doi.org/10.1016/S0168-1591(98)00188-9

Bradley, F.A., 2007. Biosecurity: Educational Programs. J. Appl. Poult. Res. 16, 77–81. https://doi.org/10.1093/japr/16.1.77

Bramble, M., Morris, D., Tolomeo, P., Lautenbach, E., 2011. Potential Role of Pet Animals in Household Transmission of Methicillin-Resistant *Staphylococcus aureus:* A Narrative Review. Vector-Borne Zoonotic Dis. 11, 617–620. https://doi.org/10.1089/vbz.2010.0025

Cannas, S., Frank, D., Minero, M., Godbout, M., Palestrini, C., 2010. Puppy behavior when left home alone: Changes during the first few months after adoption. J. Vet. Behav. 5, 94–100. https://doi.org/10.1016/J.JVEB.2009.08.009

Castelhano-Carlos, M.J., Baumans, V., 2009. The impact of light, noise, cage cleaning and in-house transport on welfare and stress of laboratory rats. Lab. Anim. 43, 311–327. https://doi.org/10.1258/la.2009.0080098

Claerebout, E., Casaert, S., Dalemans, A.C., De Wilde, N., Levecke, B., Vercruysse, J., Geurden, T., 2009. Giardia and other intestinal parasites in different dog populations in Northern Belgium. Vet Parasitol 161, 41–46. https://doi.org/10.1016/j.vetpar.2008.11.024

Coppinger, R., Zuccotti, J., 1999. Kennel Enrichment: Exercise and Socialization of Dogs. J. Appl. Anim. Welf. Sci. 2, 281–296. https://doi.org/10.1207/s15327604jaws0204_3

Coppola, C.L., Enns, R.M., Grandin, T., 2006. Noise in the Animal Shelter Environment: Building Design and the Effects of Daily Noise Exposure. J. Appl. Anim. Welf. Sci. 9, 1–7. https://doi.org/10.1207/s15327604jaws0901_1

Coyne, M.J., Burr, J.H.H., Yule, T.D., Harding, M.J., Tresnan, D.B., McGavin, D., 2001. Duration of immunity in dogs after vaccination or naturally acquired infection. Vet. Rec. 149, 509–515.

Cushing, B.S., Kramer, K.M., 2005. Mechanisms underlying epigenetic effects of early social experience: The role of neuropeptides and steroids. Neurosci. Biobehav. Rev. 29, 1089–1105. https://doi.org/10.1016/J.NEUBIOREV.2005.04.001

Czerwinski, V.H., Smith, B.P., Hynd, P.I., Hazel, S.J., 2016. The influence of maternal care on stress-related behaviors in domestic dogs: What can we learn from the rodent literature? J. Vet. Behav. 14, 52–59. https://doi.org/10.1016/J.JVEB.2016.05.003

Day, M.J., 2011. One health: the importance of companion animal vector-borne diseases. Parasites {&} Vectors 4, 49. https://doi.org/10.1186/1756-3305-4-49

Day, M.J., 2007. Immune System Development in the Dog and Cat. J. Comp. Pathol. 137, S10–S15.

Day, M.J., 2006. Vaccine side effects: Fact and fiction. Vet. Microbiol. 117, 51–58. https://doi.org/http://dx.doi.org/10.1016/j.vetmic.2006.04.017

Day, M.J., Horzinek, M.C., Schultz, R.D., Squires, R.A., 2016. WSAVA Guidelines for the vaccination of dogs and cats [WWW Document]. J. Small Anim. Pract. https://doi.org/10.1111/jsap.2_12431

De Cramer, K.G.M., Stylianides, E., van Vuuren, M., 2011. Efficacy of vaccination at 4 and 6 weeks in the control of canine parvovirus. Vet. Microbiol. 149, 126–132. https://doi.org/10.1016/J.VETMIC.2010.11.004

De Graef, E.M., Decostere, A., Devriese, L.A., Haesebrouck, F., 2004. Antibiotic Resistance among Fecal Indicator Bacteria from Healthy Individually Owned and Kennel Dogs. Microb. Drug Resist. 10, 65–69. https://doi.org/10.1089/107662904323047826

De Keuster, T., Monteny, J., Moons, C., 2015. Behaviour problems: a brief guide. British Small Animal Veterinary Association, Gloucester, UK.

De Keuster, T., Lamoureux, J., Kahn, A., 2006. Epidemiology of dog bites: A Belgian experience of canine behaviour and public health concerns. Vet. J. 172, 482–487. https://doi.org/10.1016/j.tvjl.2005.04.024

De Meester, R., Moons, C., van Bree, H., Coopman, F., 2005. Critical evaluation of the environment in Belgian dog breeding kennels during the puppies' socialization period. Vlaams Diergeneeskd. Tijdschr. 74, 364–374.

Decaro, N., Campolo, M., Desario, C., Elia, G., Martella, V., Lorusso, E., Buonavoglia, C., 2005. Maternally-derived antibodies in pups and protection from canine parvovirus infection. Biologicals 33, 261–267. https://doi.org/10.1016/J.BIOLOGICALS.2005.06.004

Dewulf, J., Van Immerseel, F., 2018. Biosecurity in animal production and veterinary medicine : From principles to practice., first edit. ed.

Dupont, S., Butaye, P., Claerebout, E., Theuns, S., Duchateau, L., Van de Maele, I., Daminet, S., 2013. Enteropathogens in pups from pet shops and breeding facilities. J. Small Anim. Pract. 54, 475–480. https://doi.org/10.1111/jsap.12119

Ebner, K., Singewald, N., 2017. Individual differences in stress susceptibility and stress inhibitory mechanisms. Curr. Opin. Behav. Sci. 14, 54–64. https://doi.org/10.1016/J.COBEHA.2016.11.016

ESCCAP, 2010. Control of Intestinal protozoa in Dogs and Cats - ESCCAP Guidelines 2nd edition [WWW Document]. URL https://www.esccap.org/guidelines/

Eterpi, M., McDonnell, G., Thomas, V., 2009. Disinfection efficacy against parvoviruses compared with reference viruses. J. Hosp. Infect. 73, 64–70. https://doi.org/10.1016/j.jhin.2009.05.016

Evermann, J.F., Wills, T.B., 2011. Immunologic development and immunization, in: Peterson, M.E., Kutzler, M.A. (Eds.), Small Animal Pediatrics: The First 12 Months of Life. Saunders/Elsevier, St Louis, pp. 104–112. https://doi.org/https://doi.org/10.1016/C2009-0-37825-3

Exner, M., Vacata, V., Hornei, B., Dietlein, E., Gebel, J., 2004. Household cleaning and surface disinfection: new insights and strategies. J. Hosp. Infect. 56, 70–75. https://doi.org/10.1016/J.JHIN.2003.12.037

Fiechter, R., Deplazes, P., Schnyder, M., 2012. Control of Giardia infections with ronidazole and intensive hygiene management in a dog kennel. Vet. Parasitol. 187, 93–98. https://doi.org/10.1016/j.vetpar.2011.12.023

Firnkes, A., Bartels, A., Bidoli, E., Erhard, M., 2017. Appeasement signals used by dogs during dog–human communication. J. Vet. Behav. 19, 35–44. https://doi.org/10.1016/J.JVEB.2016.12.012

Gácsi, M., Maros, K., Sernkvist, S., Faragó, T., Miklósi, Á., 2013. Human Analogue Safe Haven Effect of the Owner: Behavioural and Heart Rate Response to Stressful Social Stimuli in Dogs. PLoS One 8. https://doi.org/10.1371/journal.pone.0058475

Gazzano, A., Mariti, C., Notari, L., Sighieri, C., McBride, E.A., 2008. Effects of early gentling and early environment on emotional development of puppies. Appl. Anim. Behav. Sci. 110, 294–304. https://doi.org/10.1016/j.applanim.2007.05.007

Gilman, N., 2004. Sanitation in the animal shelter, in: Miller, L., Zawistowski, S. (Eds.), Shelter Medicine for Veterinarians and Staff. Blackwell Publishing, Ames, Iowa, pp. 67–78.

Gordon, J.C., Angrick, E.J., 1986. Canine parvovirus: environmental effects on infectivity. Am. J. Vet. Res. 47, 1464–7.

Grellet, A., Chastant-Maillard, S., Robin, C., Feugier, A., Boogaerts, C., Boucraut-Baralon, C., Grandjean, D., Polack, B., 2014. Risk factors of weaning diarrhea in puppies housed in breeding kennels. Prev. Vet. Med. 117, 260–265. https://doi.org/10.1016/j.prevetmed.2014.07.016

Grellet, A., De Battisti, C., Feugier, A., Pantile, M., Marciano, S., Grandjean, D., Cattoli, G., 2012a. Prevalence and risk factors of astrovirus infection in puppies from French breeding kennels, Veterinary Microbiology. https://doi.org/10.1016/j.vetmic.2011.11.012

Grellet, A., Feugier, A., Chastant-Maillard, S., Carrez, B., Boucraut-Baralon, C., Casseleux, G., Grandjean, D., 2012b. Validation of a fecal scoring scale in puppies during the weaning period. Prev. Vet. Med. 106, 315–323. https://doi.org/10.1016/j.prevetmed.2012.03.012

Harvey, N.D., Craigon, P.J., Blythe, S.A., England, G.C.W., Asher, L., 2016. Social rearing environment influences dog behavioral development. J. Vet. Behav. 16, 13–21. https://doi.org/10.1016/J.JVEB.2016.03.004

Hayes, G., 2009. Gastrointestinal foreign bodies in dogs and cats: a retrospective study of 208 cases. J. Small Anim. Pract. 50, 576–583. https://doi.org/10.1111/j.1748-5827.2009.00783.x

Hennessy, M.B., Davis, H.N., Williams, M.T., Mellott, C., Douglas, C.W., 1997. Plasma Cortisol Levels of Dogs at a County Animal Shelter. Physiol. Behav. 62, 485–490. https://doi.org/10.1016/S0031-9384(97)80328-9

Hennessy, M.B., T. Williams, M., Miller, D.D., Douglas, C.W., Voith, V.L., 1998. Influence of male and female petters on plasma cortisol and behaviour: can human interaction reduce the stress of dogs in a public animal shelter? Appl. Anim. Behav. Sci. 61, 63–77. https://doi.org/10.1016/S0168-1591(98)00179-8

Hernández-Blanco, B., Catala-López, F., 2015. Are licensed canine parvovirus (CPV2 and CPV2b) vaccines able to elicit protection against CPV2c subtype in puppies?: A systematic review of controlled clinical trials. Vet. Microbiol. 180, 1–9. https://doi.org/10.1016/J.VETMIC.2015.07.027

Hetts, S., Derrell Clark, J., Calpin, J.P., Arnold, C.E., Mateo, J.M., 1992. Influence of housing conditions on beagle behaviour. Appl. Anim. Behav. Sci. 34, 137–155. https://doi.org/10.1016/S0168-1591(05)80063-2

Hoelzer, K., Parrish, C.R., 2010. The emergence of parvoviruses of carnivores. Vet. Res. 41, 39. https://doi.org/10.1051/vetres/2010011

Horn, L., Huber, L., Range, F., 2013. The Importance of the Secure Base Effect for Domestic Dogs - Evidence from a Manipulative Problem-Solving Task. PLoS One 8, e65296. https://doi.org/10.1371/journal.pone.0065296

Hubrecht, R.C., 1993. A comparison of social and environmental enrichment methods for laboratory housed dogs. Appl. Anim. Behav. Sci. 37, 345–361. https://doi.org/10.1016/0168-1591(93)90123-7

Hurt, M.J., Croney, C., 2016. Evaluating the physical welfare of dogs in commercial breeding facilities in the United States 10181538, 150.

Iinuma, M., Yoshida, S., Fwnakoshi, M., 1991. DEVELOPMENT OF MASTICATORY MUSCLES AND ORAL BEHAVIOR FROM SUCKLING TO CHEWING IN DOGS. Camp. Eiochem. Physiol IOOA, 789–794.

Jansen, A., Schöneberg, I., Frank, C., Alpers, K., Schneider, T., Stark, K., 2005. Leptospirosis in Germany, 1962-2003. Emerg. Infect. Dis. 11, 1048–54. https://doi.org/10.3201/eid1107.041172

Kagan, L.J., Aiello, A.E., Larson, E., 2002. The role of the home environment in the transmission of infectious diseases. J. Community Health 27, 247–67.

Khoshnegah, J., Azizzadeh, M., Mahmoodi Gharaie, A., 2011. Risk factors for the development of behavior problems in a population of Iranian domestic dogs: Results of a pilot survey. Appl. Anim. Behav. Sci. 131, 123–130. https://doi.org/10.1016/J.APPLANIM.2011.02.003

Koolhaas, J.M., de Boer, S.F., Coppens, C.M., Buwalda, B., 2010. Neuroendocrinology of coping styles: Towards understanding the biology of individual variation. Front. Neuroendocrinol. 31, 307–321. https://doi.org/10.1016/j.yfrne.2010.04.001

Korbelik, J., Rand, J.S., Morton, J.M., 2011. Comparison of early socialization practices used for litters of small-scale registered dog breeders and nonregistered dog breeders. J. Am. Vet. Med. Assoc. 239, 1090–1097. https://doi.org/10.2460/javma.239.8.1090

Kuhne, F., Hössler, J.C., Struwe, R., 2012. [Affective behavioural responses by dogs to tactile human-dog interactions]. Berl. Munch. Tierarztl. Wochenschr. 125, 371–8.

Kuhne, F., Hößler, J.C., Struwe, R., 2014. Emotions in dogs being petted by a familiar or unfamiliar person: Validating behavioural indicators of emotional states using heart rate variability. Appl. Anim. Behav. Sci. 161, 113–120. https://doi.org/10.1016/J.APPLANIM.2014.09.020

Kustritz, M.V.R., 2005. Reproductive behavior of small animals. Theriogenology 64, 734–746. https://doi.org/10.1016/J.THERIOGENOLOGY.2005.05.022

Lawler, D.F., 2008. Neonatal and pediatric care of the puppy and kitten. Theriogenology 70, 384–392. https://doi.org/10.1016/j.theriogenology.2008.04.019

Lefebvre, D., Giffroy, J.-M., Diederich, C., 2009. Cortisol and behavioral responses to enrichment in military working dogs. J. Ethol. 27, 255–265. https://doi.org/10.1007/s10164-008-0114-z

Loveridge, G.., 1998. Environmentally enriched dog housing. Appl. Anim. Behav. Sci. 59, 101–113. https://doi.org/10.1016/S0168-1591(98)00125-7

Mariti, C., Carlone, B., Ricci, E., Sighieri, C., Gazzano, A., 2014. Intraspecific attachment in adult domestic dogs (Canis familiaris): Preliminary results. Appl. Anim. Behav. Sci. 152, 64–72. https://doi.org/10.1016/J.APPLANIM.2013.12.002

Mariti, C., Gazzano, A., Moore, J.L., Baragli, P., Chelli, L., Sighieri, C., 2012. Perception of dogs' stress by their owners. J. Vet. Behav. 7, 213–219. https://doi.org/10.1016/J.JVEB.2011.09.004

Martínez-Moreno, F.J., Hernández, S., López-Cobos, E., Becerra, C., Acosta, I., Martínez-Moreno, A., 2007. Estimation of canine intestinal parasites in Córdoba (Spain) and their risk to public health. Vet. Parasitol. 143, 7–13. https://doi.org/10.1016/J.VETPAR.2006.08.004

Mason, G., Clubb, R., Latham, N., Vickery, S., 2007. Why and how should we use environmental enrichment to tackle stereotypic behaviour? Appl. Anim. Behav. Sci. 102, 163–188. https://doi.org/10.1016/J.APPLANIM.2006.05.041

McMillan, F.D., Duffy, D.L., Serpell, J.A., 2011. Mental health of dogs formerly used as 'breeding stock' in commercial breeding establishments. Appl. Anim. Behav. Sci. 135, 86–94. https://doi.org/http://dx.doi.org/10.1016/j.applanim.2011.09.006

Meints, K., de Keuster, T., 2009. Brief Report: Don't Kiss a Sleeping Dog: The First Assessment of "The Blue Dog" Bite Prevention Program. J. Pediatr. Psychol. 34, 1084–1090. https://doi.org/10.1093/jpepsy/jsp053

Menor-Campos, D.J., Molleda-Carbonell, J.M., López-Rodríguez, R., 2011. Effects of exercise and human contact on animal welfare in a dog shelter. Vet. Rec. 169, 388. https://doi.org/10.1136/vr.d4757

Merola, I., Prato-Previde, E., Marshall-Pescini, S., 2012. Dogs' Social Referencing towards Owners and Strangers. PLoS One 7, e47653. https://doi.org/10.1371/journal.pone.0047653

Mickelsen, W.D., Memon, M.A., Anderson, P.B., Freeman, D.A., 1993. The relationship of semen quality to pregnancy rate and litter size following artificial insemination in the bitch. Theriogenology 39, 553–560. https://doi.org/10.1016/0093-691X(93)90397-N

Milani, C., Corrò, M., Drigo, M., Rota, A., 2012. Antimicrobial resistance in bacteria from breeding dogs housed in kennels with differing neonatal mortality and use of antibiotics. Theriogenology 78, 1321–1328. https://doi.org/https://doi.org/10.1016/j.theriogenology.2012.05.033

Mircean, V., Györke, A., Cozma, V., 2012. Prevalence and risk factors of Giardia duodenalis in dogs from Romania. Vet. Parasitol. 184, 325–329. https://doi.org/10.1016/J.VETPAR.2011.08.022

Moore, G.E., Guptill, L.F., Ward, M.P., Glickman, N.W., Faunt, K.K., Lewis, H.B., Glickman, L.T., 2005. Adverse events diagnosed within three days of vaccine administration in dogs. J. Am. Vet. Med. Assoc. 227, 1102–1108. https://doi.org/10.2460/javma.2005.227.1102

Münnich, A., 2008. The pathological newborn in small animals: the neonate is not a small adult. Vet. Res. Commun. 32, 81–85. https://doi.org/10.1007/s11259-008-9096-0

Münnich, A., Lübke-Becker, A., 2004. Escherichia coli infections in newborn puppies—clinical and epidemiological investigations. Theriogenology.

Neumann, I.D., Krömer, S.A., Bosch, O.J., 2005. Effects of psycho-social stress during pregnancy on neuroendocrine and behavioural parameters in lactation depend on the genetically determined stress vulnerability. Psychoneuroendocrinology 30, 791–806. https://doi.org/10.1016/J.PSYNEUEN.2005.03.008

Nielen, A.L., van der Gaag, I., Knol, B.W., Schukken, Y.H., 1998. Investigation of mortality and pathological changes in a 14-month birth cohort of boxer puppies. Vet. Rec. 142, 602–6. https://doi.org/10.1136/VR.142.22.602

Okkens, A.C., Hekerman, T.W.M., de Vogel, J.W.A., van Haaften, B., 1993. Influence of litter size and breed on variation in length of gestation in the dog. Vet. Q. 15, 160–161. https://doi.org/10.1080/01652176.1993.9694397

Olson, M., Goh, J., Phillips, M., 1999. Giardia cyst and Cryptosporidium oocyst survival in water, soil, and cattle feces. J.

Overgaauw, P.A., van Knapen, F., 2013. Veterinary and public health aspects of Toxocara spp. Vet Parasitol 193, 398–403. https://doi.org/10.1016/j.vetpar.2012.12.035

Patel, J.R., Heldens, J.G.M., 2009. Review of companion animal viral diseases and immunoprophylaxis. Vaccine 27, 491–504. https://doi.org/http://dx.doi.org/10.1016/j.vaccine.2008.11.027

Pollock, R. V, Carmichael, L.E., 1982. Maternally derived immunity to canine parvovirus infection: transfer, decline, and interference with vaccination. J. Am. Vet. Med. Assoc. 180, 37–42.

Previde, E.P., Ghirardelli, G., Marshall-Pescini, S., Valsecchi, P., 2009. Intraspecific attachment in domestic puppies (Canis familiaris). J. Vet. Behav. Clin. Appl. Res. 4, 89–90. https://doi.org/10.1016/J.JVEB.2008.10.002

Protopopova, A., 2016. Effects of sheltering on physiology, immune function, behavior, and the welfare of dogs. Physiol. Behav. 159, 95–103.

Pullen, A.J., Merrill, R.J.N., Bradshaw, J.W.S., 2012. Habituation and dishabituation during object play in kennel-housed dogs. Anim. Cogn. 15, 1143–1150. https://doi.org/10.1007/s10071-012-0538-2

Pullen, A.J., Merrill, R.J.N., Bradshaw, J.W.S., 2010. Preferences for toy types and presentations in kennel housed dogs. Appl. Anim. Behav. Sci. 125, 151–156. https://doi.org/10.1016/j.applanim.2010.04.004

Rankin, C.H., Abrams, T., Barry, R.J., Bhatnagar, S., Clayton, D.F., Colombo, J., Coppola, G., Geyer, M.A., Glanzman, D.L., Marsland, S., McSweeney, F.K., Wilson, D.A., Wu, C.-F., Thompson, R.F., 2009. Habituation revisited: An updated and revised description of the behavioral characteristics of habituation. Neurobiol. Learn. Mem. 92, 135–138. https://doi.org/10.1016/J.NLM.2008.09.012

Rika-Heke, T., Kelman, M., Ward, M.P., 2015. The relationship between the Southern Oscillation Index, rainfall and the occurrence of canine tick paralysis, feline tick paralysis and canine parvovirus in Australia. Vet. J. 205, 87–92. https://doi.org/10.1016/J.TVJL.2015.03.012

Robertson, I.D., Irwin, P.J., Lymbery, A.J., Thompson, R.C.A., 2000. The role of companion animals in the emergence of parasitic zoonoses. Int. J. Parasitol. 30, 1369–1377. https://doi.org/10.1016/S0020-7519(00)00134-X

Ronsse, V., Verstegen, J., Thiry, E., Onclin, K., Aeberle, C., Brunet, S., Poulet, H., 2005. Canine herpesvirus-1 (CHV-1): clinical, serological and virological patterns in breeding colonies. Theriogenology 64, 61–74. https://doi.org/10.1016/j.theriogenology.2004.11.016

Rota, A., Milani, C., Corrò, M., Drigo, I., Börjesson, S., 2013. Misuse of Antimicrobials and Selection of Methicillin-Resistant Staphylococcus pseudintermedius Strains in Breeding Kennels: Genetic Characterization of Bacteria After a Two-year Interval. Reprod. Domest. Anim. 48, 1–6. https://doi.org/10.1111/j.1439-0531.2012.02012.x

Rota, A., Milani, C., Drigo, I., Drigo, M., Corrò, M., 2011. Isolation of methicillin-resistant Staphylococcus pseudintermedius from breeding dogs. Theriogenology 75, 115–121. https://doi.org/10.1016/j.theriogenology.2010.07.016

Sapolsky, R.M., Romero, L.M., Munck, A.U., 2000. How Do Glucocorticoids Influence Stress Responses? Integrating Permissive, Suppressive, Stimulatory, and Preparative Actions [1]. Endocr. Rev. 21, 55–89. https://doi.org/10.1210/edrv.21.1.0389

Scarlett, J.M., Saidla, J.E., Pollock, R. V, 1994. Source of acquisition as a risk factor for disease and death in pups. J. Am. Vet. Med. Assoc. 204, 1906–13.

Schäfer-Somi, S., Spergser, J., Breitenfellner, J., Aurich, J.E., 2003. Bacteriological status of canine milk and septicaemia in neonatal puppies--a retrospective study. J. Vet. Med. B. Infect. Dis. Vet. Public Health 50, 343–6.

Schalamon, J., Ainoedhofer, H., Singer, G., 2006. Analysis of dog bites in children who are younger than 17 years. Pediatrics.

Schultz, R.D., Thiel, B., Mukhtar, E., Sharp, P., Larson, L.J., 2010. Age and long-term protective immunity in dogs and cats. J Comp Pathol 142 Suppl, S102-8. https://doi.org/10.1016/j.jcpa.2009.10.009

Sciensano, 2019. Risico's en gevaren Rabies [WWW Document]. URL https://www.sciensano.be/nl/gezondheidsonderwerpen/rabies/risicos-en-gevaren (accessed 3.29.19).

Seksel, K., Mazurski, E.E.J., Taylor, A., 1999. Puppy socialisation programs: short and long term behavioural effects. Appl. Anim. Behav. Sci. 62, 335–349. https://doi.org/http://dx.doi.org/10.1016/S0168-1591(98)00232-9

Shaw, S.E., Langton, D.A., Hillman, T.J., 2009. Canine leishmaniosis in the United Kingdom: a zoonotic disease waiting for a vector? Vet Parasitol 163. https://doi.org/10.1016/j.vetpar.2009.03.025

Smith, A.F., Rock, M., Neumann, N., Massolo, A., 2015. Urban park-related risks for Giardia spp. infection in dogs. Epidemiol. Infect. 143, 3277–3291. https://doi.org/10.1017/S0950268815000400

Socialising puppies and dogs | Blue Cross [WWW Document], URL https://www.bluecross.org.uk/pet-advice/socialising-puppies-and-dogs (accessed 2.11.19).

Sokolow, S.H., Rand, C., Marks, S.L., Drazenovich, N.L., Kather, E.J., Foley, J.E., 2005. Epidemiologic evaluation of diarrhea in dogs in an animal shelter. Am. J. Vet. Res. 66, 1018–1024. https://doi.org/10.2460/ajvr.2005.66.1018

Solano-Gallego, L., Sainz, Á., Roura, X., Estrada-Peña, A., Miró, G., 2016. A review of canine babesiosis: the European perspective 9, 336. https://doi.org/10.1186/s13071-016-1596-0

Solomon, R.L., Kamin, L.J., Wynne, L.C., 1953. Traumatic avoidance learning: the outcomes of several extinction procedures with dogs. J. Abnorm. Soc. Psychol. 48, 291–302. https://doi.org/10.1037/h0058943

Spangenberg, E.M.F., Björklund, L., Dahlborn, K., 2006. Outdoor housing of laboratory dogs: Effects on activity, behaviour and physiology. Appl. Anim. Behav. Sci. 98, 260–276. https://doi.org/10.1016/J.APPLANIM.2005.09.004

STATBEL, 2018. Kadastrale statistiek van het gebouwenpark [WWW Document]. URL https://bestat.statbel.fgov.be/bestat/crosstable.xhtml?view=dade64a8-39c8-498e-9736-16a27ef618a0 (accessed 2.11.19).

Stepita, M.E., Bain, M.J., Kass, P.H., 2013. Frequency of CPV Infection in Vaccinated Puppies that Attended Puppy Socialization Classes. J. Am. Anim. Hosp. Assoc. 49, 95–100. https://doi.org/10.5326/JAAHA-MS-5825

Stoffel, M.H., Friess, A.E., Hartmann, S.H., 2000. Ultrastructural evidence of transplacental transport of immunoglobulin G in bitches. J. Reprod. Fertil. 118, 315–26.

Taylor, K., Mills, D., 2007. The effect of the kennel environment on canine welfare: a critical review of experimental studies. Anim. WELFARE-POTTERS BAR.

Terpstra, F.G., van den Blink, A.E., Bos, L.M., Boots, A.G.C., Brinkhuis, F.H.M., Gijsen, E., van Remmerden, Y., Schuitemaker, H., van 't Wout, A.B., 2007. Resistance of surface-dried virus to common disinfection procedures. J. Hosp. Infect. 66, 332–338. https://doi.org/10.1016/j.jhin.2007.05.005

Thiry, E., Horzinek, M.C., 2007. Vaccination guidelines: a bridge between official requirements and the daily use of vaccines. Rev. Sci. Tech. 26, 511–517.

Tiira, K., Lohi, H., 2015. Early Life Experiences and Exercise Associate with Canine Anxieties. PLoS One 10, e0141907. https://doi.org/10.1371/journal.pone.0141907

Toman, M., Faldyna, M., Knotigova, P., Pokorova, D., Sinkora, J., 2002. Postnatal development of leukocyte subset composition and activity in dogs. Vet. Immunol. Immunopathol. 87, 321–326. https://doi.org/10.1016/S0165-2427(02)00058-2

Tuchscherer, M., Kanitz, E., Otten, W., Tuchscherer, A., 2002. Effects of prenatal stress on cellular and humoral immune responses in neonatal pigs. Vet. Immunol. Immunopathol. 86, 195–203. https://doi.org/10.1016/S0165-2427(02)00035-1

Twark, L., Dodds, W.J., 2000. Clinical use of serum parvovirus and distemper virus antibody titers for determining revaccination strategies in healthy dogs. J. Am. Vet. Med. Assoc. 217, 1021–1024. https://doi.org/10.2460/javma.2000.217.1021

Valsecchi, P., Pattacini, O., Beretta, V., Bertozzi, J., Zannoni, S., Viggiani, R., Accorsi, P.A., 2007. 13: Effects of a human social enrichment program on behavior and welfare of sheltered dogs. J. Vet. Behav. 2, 88–89. https://doi.org/10.1016/J.JVEB.2007.04.017

Vas, J., Topál, J., Gácsi, M., Miklósi, Á., Csányi, V., Miklósi, A., Csányi, V., 2005. A friend or an enemy? Dogs' reaction to an unfamiliar person showing behavioural cues of threat and friendliness at different times. Appl. Anim. Behav. 94, 99–115. https://doi.org/10.1016/j.applanim.2005.02.001

Wells, D.L., 2004. A review of environmental enrichment for kennelled dogs, Canis familiaris. Appl. Anim. Behav. Sci. 85, 307–317. https://doi.org/10.1016/j.applanim.2003.11.005

Westgarth, C., Christley, R.M., Pinchbeck, G.L., Gaskell, R.M., Dawson, S., Bradshaw, J.W.S., 2010. Dog behaviour on walks and the effect of use of the leash. Appl. Anim. Behav. Sci. 125, 38–46. https://doi.org/10.1016/J.APPLANIM.2010.03.007

WHO | Vaccines, 2017. . WHO.

Winters, W.D., 1981. Time dependent decreases of maternal canine virus antibodies in newborn pups. Vet. Rec. 108, 295–9. https://doi.org/10.1136/VR.108.14.295

Xong, Y., Meng, Q. shi, Gao, J., Tang, X. fang, Zhang, H. fu, 2017. Effects of relative humidity on animal health and welfare. J. Integr. Agric. 16, 1653–1658. https://doi.org/10.1016/S2095-3119(16)61532-0

Yao, P.J., Stephenson, N., Foley, J.E., Toussieng, C.R., Farver, T.B., Sykes, J.E., Fleer, K.A., 2015. Incidence rates and risk factors for owner-reported adverse events following vaccination of dogs that did or did not receive a Leptospira vaccine. J. Am. Vet. Med. Assoc. 247, 1139–1145. https://doi.org/10.2460/javma.247.10.1139

SUMMARY

The increasing popularity of dogs has provoked a shift in breeding practices, giving rise to a societal debate on intensive dog breeding. The environment shaped by the breeder plays an important role in the behavioural development of puppies, and adverse situations at the breeder can result in behavioural impairment later in life. There exists great variability between different dog breeding facilities, and inferiority of puppies originating from intensive breeding facilities is suggested. To date however, no systematic investigation of the various breeding systems has been performed.

The environment at the breeder can harbour various pathogens that will impair the puppies' health and welfare or represent a zoonotic risk, and puppies and pregnant dams are more at risk to contract a disease. Appropriate strategies to maintain biosecurity, that also take into account environmental factors, are of utmost importance to limit disease. However, guidelines on how to improve socialisation and environmental learning practices without compromising the welfare and health of puppies in intensive dog breeding are currently lacking.

It is crucial to characterise the current husbandry conditions at different breeder types that may influence the health, hygiene, socialisation, environmental learning, and welfare. Subsequently, measuring behavioural and health outcomes in relation to the environment will provide potential areas of improvement. Finally, an evaluation of pet dog behaviour shortly after homing will help to assess the differences between dog breeder types, as perceived by the owner after the sale.

To assess all factors that influence the behavioural development and the health of puppies during their time at the breeder a multidisciplinary approach is required.

First, we conducted a cross-sectional study, that revealed considerable variability in environment among dog breeders. Small-scale breeders, and especially occasional breeders (less than 10 adult dogs on site) provided most enrichment, both social and nonsocial, by, for instance, providing more outdoor access for pregnant dams and puppies or by providing access to visitors more freely. Environmental stimuli were less controlled in occasional breeders, raising the debate about quantity versus quality of stimuli at a young age. Large-scale breeders declared to screen potential owners less intensely and the time to advise them was limited.

Second, we conducted a cross-sectional study of health management and biosecurity measures in 102 Belgian dog breeding facilities. Veterinary prophylactic protocols (i.e., vaccination, endoparasite control, ectoparasitic treatments) were mostly highly implemented across all breeder categories. 13.8% of all visited breeders reported to administer antimicrobials to each female post-partum and 10.3% reported to treat all puppies, or at least of one breed, systematically with antimicrobials. Large-scale breeders reported to employ staff more frequently, and appeared to be more familiar with the principles of biosecurity. Compared to small-scale breeders, they reported to apply disinfection more often at the adult dogs and at the maternity ward. They also reported to apply hygienic measures more often at the maternity ward and the nursery and had a tendency to apply hygienic measures more often at the adult dogs and to quarantine newly acquired dogs more often compared to small-scale breeders. Nonetheless, a moderate knowledge and use of disinfection was recorded. Pet dogs were found to be present at all breeder categories, breaking the rules of compartmentalisation.

Third, we conducted a longitudinal study where 107 puppies from 23 litters in their pens were observed during rest and while being subjected to a nonsocial and social novel disturbance, one week before homing, at the mean age of 9.4 weeks (standard deviation ± 2.9). Puppies from commercial breeders showed more exploration activity compared to puppies from breeders of other categories in all contexts. These puppies also spent proportionally more time investigating the novel object compared to puppies from merchants and had more interactions with littermates during the novel object disturbance. No differences in the frequency of social conflicting signals in any of the contexts could be identified between breeders.

Last, a cross-sectional study of newly acquired puppies was conducted in 20 veterinary practices. Puppies were assessed on their behaviour, both by the veterinarian and by the owner, and on general health. Puppies from dog merchants were more often presented to veterinarians because of illness compared to puppies from occasional breeders. Puppy age was not significantly associated with any items from the behaviour assessment by the veterinarian. Puppies originating from commercial breeders were scored as less fearful than puppies bred by occasional breeders, and owners of puppies originating from occasional breeders also scored their puppy higher on stranger-directed aggression and fear, and on nonsocial fear than owners of puppies originating from dog merchants. Puppies from

occupational breeders were found to be more passive compared to dogs from occasional breeders, commercial breeders and merchants.

The results of this research indicate that there is substantial room for improvement in hygiene and disease management across all categories of breeders. Although prophylactic treatments are widely applied, an increase in knowledge of current scientific recommendations is advisable. Additionally, a higher awareness for control over aversive stimuli for pregnant dams and puppies is desirable, especially in small-scale breeders. Large-breeders could make more efforts to provide enriched environment and diverse positive social interactions, without compromising the biosecurity. The results also indicate that the breeder category has an effect on behavioural tendencies, and that puppies from commercial breeders are more proactive. More research is needed to determine the influence of this behavioural tendency of puppies on the behaviour later in life.

NEDERLANDSTALIGE SAMENVATTING

De toenemende populariteit van de hond heeft geleid tot een verschuiving in kweekpraktijken, wat een maatschappelijk debat over intensieve hondenkwekerij op gang heeft gebracht. De omgeving die door de hondenkweker gevormd wordt, vormt een belangrijk onderdeel van de gedragsontwikkeling van puppy's en nadelige situaties bij de kweker kunnen leiden tot gedragsstoornissen in het latere leven van de hond. Er zijn grote verschillen tussen de verschillende types hondenkwekers en er wordt gesuggereerd dat de puppy's afkomstig van intensieve kweekbedrijven inferieur zijn. Tot op heden is er echter geen systematisch onderzoek van de verschillende types kwekers uitgevoerd.

De omgeving bij de kweker kan tevens verschillende ziekteverwekkers herbergen die de gezondheid en het welzijn van de puppy's schaden of een zoönotisch risico vormen. Bovendien lopen puppy's en drachtige teven meer risico om een ziekte op te lopen. Geschikte strategieën om de bioveiligheid te handhaven, die tevens rekening houden met de omgevingsfactoren, zijn van groot belang om ziektes te beperken. Echter momenteel ontbreken richtlijnen om de socialisatie en het omgevings-gerelateerd leren van puppy’s te stimuleren zonder hun welzijn en gezondheid in gevaar te brengen

Het is van cruciaal belang om de omstandigheden bij de verschillende types kwekers, die van invloed kunnen zijn op de gezondheid, hygiëne, socialisatie, omgevings-gerelateerd leren en welzijn, te onderscheiden. De beoordeling van het gedrag en de gezondheid van de puppy’s in relatie tot de omgeving zal potentiële verbeterpunten kunnen bieden. Ten slotte zal een evaluatie van het gedrag van de hond kort na aanschaf, zoals waargenomen door de eigenaar, helpen om de verschillen tussen de types fokker in kaart te brengen.

Om alle factoren die de gedragsontwikkeling en de gezondheid van puppy's beïnvloeden tijdens hun verblijf bij de kweker te kunnen beoordelen is bijgevolg een multidisciplinaire aanpak vereist.

Eerst hebben we een cross-sectionele studie uitgevoerd, die een aanzienlijke variabiliteit in de omgeving bij hondenkwekers aan het licht bracht. Kleinschalige kwekers, met name occasionele kwekers (minder dan 10 volwassen honden ter plaatse), voorzagen de meeste verrijking, zowel sociaal als niet-sociaal. Bijvoorbeeld door drachtige teven en puppy's meer toegang naar buiten te bieden, of door bezoekers vrije toegang te verlenen. Omgevings-

gerelateerde stimuli werden minder gecontroleerd bij occasionele kwekers, wat het debat over kwantiteit versus kwaliteit van stimuli op jonge leeftijd doet oplaaien. Grootschalige kwekers verklaarden dat potentiële eigenaren minder intensief gescreend werden en de tijd om hen te adviseren was beperkt.

Ten tweede hebben we een cross-sectionele studie uitgevoerd naar gezondheidsmanagement en bioveiligheidsmaatregelen in 102 Belgische hondenkwekerijen. Veterinaire profylactische protocollen (d.w.z. vaccinatie, endoparasitaire en ectoparasitaire behandelingen) werden ruim toegepast bij alle types kwekers. Daarnaast verklaarde 13,8% van alle bezochte kwekers antimicrobiële middelen toe te dienen aan elke teef postpartum en 10,3% meldde dat ze alle puppy's, of tenminste allen van één ras, systematisch met antimicrobiële middelen behandelen. Grootschalige kwekers verklaarden vaker personeel in dienst te nemen en bleken meer vertrouwd te zijn met de principes van bioveiligheid. In vergelijking met kleinschalige kwekers verklaarden ze vaker desinfectie en hygiënische maatregelen toe te passen bij de volwassen honden en in de materniteit. Ze verklaarden ook vaker hygiënische maatregelen to te passen in de materniteit en bij de puppy's en hadden de neiging om vaker hygiënische maatregelen toe te passen bij de volwassen honden en om nieuw verworven honden in quarantaine te plaatsen in vergelijking met kleinschalige kwekers. Desalniettemin werd slechts een gematigde kennis en gebruik van desinfectie geconstateerd. Bij alle types kwekers werd de aanwezigheid van huisdieren geconstateerd, waarbij de regels van compartimentering werden overtreden.

Ten derde hebben we een longitudinale studie uitgevoerd waarbij 107 pups van 23 nesten in hun hok werden waargenomen in rust en terwijl ze werden blootgesteld aan een sociale (onbekend persoon) en een niet-sociale (onbekend object) verstoring. Dit gebeurde één week voor aanschaf, op de gemiddelde leeftijd van 9,4 weken (standaarddeviatie ± 2,9). Puppy's van commerciële kwekers vertoonden meer exploratieve activiteit vergeleken met puppy’s van alle andere types kwekers. Deze puppy's besteedden verhoudingsgewijs ook meer tijd aan het onderzoeken van het nieuwe object in vergelijking met puppy's van handelaren en hadden meer interacties met nestgenoten tijdens de niet-sociale verstoring. Er konden geen verschillen worden vastgesteld in de frequentie van sociaal conflicterende signalen in een van de contexten tussen de types kwekers.

Als laatste werd er een cross-sectionele studie van juist aangeschafte puppy's uitgevoerd in 20 dierenartsen praktijken. Puppy's werden beoordeeld op gedrag, zowel door de dierenarts als door de eigenaar; en op gezondheid. Puppy's van handelaren werden vaker ter consult aangeboden vanwege ziekte in vergelijking met puppy's van occasionele kwekers. De leeftijd van de puppy was niet significant gerelateerd aan onderdelen van de gedragsbeoordeling door de dierenarts. Puppy's afkomstig van commerciële kwekers werden als minder angstig beoordeeld dan puppy's die door occasionele kwekers werden verkocht, en eigenaren van puppy's afkomstig van occasionele kwekers scoorden hun puppy hoger op agressie en angst voor vreemden en op niet-sociale angst, dan eigenaren van puppy's afkomstig van handelaren. Puppy's van occasionele kwekers bleken meer passief te zijn in vergelijking met puppy's van alle andere types kwekers.

De resultaten van dit multidisciplinaire onderzoek tonen aan dat er bij alle types kwekers aanzienlijk ruimte voor verbetering is op het gebied van hygiëne en gezondheidsmanagement. Hoewel profylactische behandelingen ruim worden toegepast, is een verbetering van de kennis van de huidige wetenschappelijke aanbevelingen aan te raden. Daarnaast is een verhoogd bewustzijn voor de controle van aversieve stimuli bij drachtige teven en puppy's wenselijk, vooral bij kleinschalige kwekers.

Grootschalige kwekers zouden meer inspanningen kunnen leveren om een verrijkte omgeving en diverse positieve sociale interacties te bieden, zonder de bioveiligheid in gevaar te brengen. De resultaten geven ook aan dat het type kweker een effect heeft op gedragstendensen en dat puppy's van commerciële kwekers meer proactief zijn. Meer onderzoek is nodig om te bepalen wat de invloed is van de gedragstendens van een puppy op het gedrag later in het leven.

ACKNOWLEDGEMENTS

Five summers ago, the advert for a PhD research published in the SAVAB Flanders newsletter caught my attention. The black-on-white letters on my computer screen resonated in my head… I started this journey unbiased and with no particular expectations but with the clear conviction of making a difference by contributing to animal welfare. And boy, it was an incredible journey. Since 2014, a myriad of people have contributed to what has come to an end today, at the sunset of the historical summer of 2019. I could not have completed my research without the support of all these wonderful people.

First, I would like to acknowledge my promotors, all five of them, for giving me this opportunity and providing me with assistance and guidance while I navigated through this process to complete the PupWelfare research project.

Christel, I really appreciate your honesty, directness and fire when debating over results. **Claire**, thank you for the broad knowledge you brought to the project and your pragmatic approach of the issues that crossed it. Also, I wish to thank both of you very much for your patience and help in the administrative jungle of a joint PhD. **Jeroen**, your contributions were always relevant and to the point. On a regular basis, your exemplary decisiveness provided peace in this project. Thank you for this! **Tiny**, my gratefulness for your contributions is immense. Your critical mind challenged me to reflect more, broaden and deepen my knowledge and to question every result. **Etienne**, your eye for detail and your ability to pick the one mistake out of a text or presentation surprised me every time.

It has been a steep learning curve. Regularly, all of you challenged me to restructure and explain my thoughts. As a person with A.D.D., the waterfall of thoughts is a daily constant and in many ways, being able to finish such a research project has been my greatest achievement yet. This PhD has taught me to deal with my own limitations, forced me to focus, showed me the importance of stakeholder management and how to deal with more work than time available and thus to prioritize.

I would like to thank the president, the secretary and all the members of the **jury** for their time in reviewing my dissertation and providing valuable feedback. Your ability to pinpoint the problem sometimes flabbergasted me and motivated me to think even further.

I also want to thank the **Federal Public Service of Public Health, Food Safety and Environment** of the Belgian government, and the **Department of Environment** of the Flemish Government to provide financial support for this research and the federal and regional governments for prevailing me of the hassle of paying taxes during four years. As an extension, I wish to acknowledge the members of the project **Guidance Committee** for not sparing their effort to provide input and the necessary reflection of the various parts of the project and the yearly advancements.

I'm grateful to my former manager, **Claude**, for providing me with the freedom to start my PhD and to share with me which aspects of a PhD matter in the corporate world. In extent, I wish to thank my current **colleagues** at **Royal Canin** for supporting me during these months in which I tried by all means to combine to uncombinable. I'm very thankful to my manager, **Maarten**, for acknowledging how important this project was to me and for providing me with the time and the freedom to knot all ends together. This PhD has been finalised thanks to your support, patience and flexibility.

I also want to thank all the people of the various Belgian Canine breeding organisations (especially **Ani-ZOO** and **KMSH**) and the individual **dog breeders** who provided support and actively participated, shared, followed or contributed in one way or another to this research project.

I wish to acknowledge the **veterinarians** who participated in this project by piloting the questionnaires, by contributing to the data collection and by sharing the project. Special thank you to **VDWE** and **SAVAB**-Flanders, without you I would not have heard of this wonderful project. I want to acknowledge **UPV** and **Dierenartsenwereld** for promoting the project and sharing our advertisement.

Thank you **Hilde** and **Eline W.**, for your wise contribution to the book chapter in biosecurity. Thank you **Ann** and **Dominique**, for providing me with the opportunity to share knowledge with a different audience. Thank you **William** and **Flàvia** for your scientific contribution.

I also wish to thank my colleagues at Namur University: **Damien, Isabelle, Laëtitia, Sophie, Frédérique, Marianne, Srdan, François, Chantal, Yves, Benoît, Nathalie**, the **interns** and all who crossed my path. I particularly enjoyed our lunches during which we could discuss all kinds of subjects, ranging from virology, laboratory techniques and molecular biology to tourism and parenthood. **Damien**, I'm grateful for showing me the ins and outs at UNamur, for sharing your knowledge about publications and about life as a PhD. Also, thank you for sharing your office with me, to me it meant a great deal because it was the first time in my career that I received a dedicated office. **Rian**: your enthusiasm and experience provided me with the energy that was needed at the onset, thank you very much. Your help in the initial development by providing me with the tools and the contacts certainly gave me a head start.

I also wish to thank the colleagues from Ghent University for providing moral support and sharing difficulties. The "e-pig team" (or is it *epic*? *Epi-Pig*?): **Bert, Nele, Moniek, Filip, Steven, Merel, Marilena, Ileas, Marjolein, Bo, Evelien, Sarah, Annelies, Linda, Ioannis**, and all others. Your daily comments on our What'sApp group continuously entertained me and I enjoyed the moments we spent together. A special mention for the very interesting people that study genetics. Yes you, who share a lot of our laboratory equipment, offices and ideas but are definitely from a totally different team: **Francis**, and your critical yet open mind, **Arlieke, Evy, Hanne, Joyca, Tom, Sean** ["Shawn" in phonetics], **Mario, Dominique, Linda, Ruben** and the ones I may have omitted: thank you! **Bart**: the eternal optimist, it was a pleasure to debate with you during our lunches and nostalgically remember the old days as students. Colleagues of nutrition, and especially **Myriam** and **Amy**, thank you for providing me with the opportunity to maintain some of my veterinary skills by allowing me to examine the beagles.

Most importantly, my very dear colleagues**, Ana, Glynnis** and **Jeroen**. When I arrived in the Heidestraat, I started by dusting off an old classroom, with I filled with ideas, thoughts and eventually a lot of frustrations. **Glynnis**, I will remember your immense kindness. I learned a lot from your ability to prioritize and how to make steady advancements. **Jeroen**, you pulled me into the most various and interesting conversations. I particularly enjoyed your broad knowledge and your great sense of humour. **Ana**, your venue was the biggest enrichment (social and non-social) I only could have hoped for. You transformed this office into a place

where we could share secrets, ventilate frustration, help each other in diplomatic emails, in analysis, in understanding behaviour, in motivating each other and, not least of all, our infamous “after work drink” Fridays. You provided me with a second home that greatly contributed to my energy level and motivation.

I’m very grateful to my family: ***Maman**, tu nous as accueillis et tu t’es investie sans limites pour nous fournir le chez-nous dont nous avions besoin. Cela a fait LA différence. Je ne pourrai jamais exprimer toute ma gratitude avec des mots. **Papa**, depuis le berceau, tu as insisté sur l'importance de comprendre comment le monde fonctionne et comment tout interagit. Toutes les heures que nous avons passées ensemble à discuter entre les consultations m’ont apprits que les pensées sont ce qu’il y a de plus puissant. Tu m’as aidé à prendre les bonnes décisions et tu m'as incité à poursuivre la carrière de vétérinaire au lieu de prendre un chemin différent. Sans ton sens de la réalité, je n'aurais jamais osé penser à commencer un doctorat.* **Justine** and **Jon**, thank you so much for your encouragement and continued support over the years. The ability to discuss methodology and statistics from a different perspective helped me move through the deepest pit of this PhD. **Charles-Edouard**, my dear brother, you were there at the onset and I sincerely regret that we did not finish this together.

I wish to thank my Kids, **Élodie-Charlotte** and **Marc-Aurèle**, for your daily smiles and cuddles, being so sweet and help me forget the stress of my PhD. Your patience has often been tested by all the evenings, weekend and holidays where daddy did not have time for you. Yet, you provided me the nicest gift during this PhD: the ability to take distance and recharge.

I could start this acknowledgement with you, my dear wife, **Eline**, for your unconditional and infinite support. Because our life is our fairy tale, I preferred to end with mentioning your name. You contributed to each and every step of this PhD, from the onset to today’s presentation. You pulled and pushed when needed. You were the assistant, confidante and associate I could only wish for. You spend weeks in rereading, rewording, correcting and ameliorating every single text. Meanwhile, you proved to be an exemplary mother, passionate wife and you conducted your own PhD. My gratitude is only equaled by the love I have for you.
And they lived happily ever after…

CURRICULUM VITAE

Pierre-Alexandre Dendoncker was born on June 16th, 1984 in Brussels, Belgium.

From the cradle, he heard about Veterinary Medicine from his father and Pierre-Alexandre grew up surrounded by animals of all kinds. In primary school, he held passionate lectures about several aspects of animals, for instance the heredity of coat colour and was called “the little professor” by his teachers. In 2003, he started veterinary school and took this opportunity to broaden his horizons towards community management and leadership. He graduated in 2011 as a companion animal veterinarian from Ghent University, Belgium.

For two years, he worked in the Netherlands as a practicing veterinarian in a first- and second-line clinic for small animals and occasionally zoo animals. In 2013 he returned to Belgium and enrolled as territory manager BeLux for Ceva Santé Animale, a veterinary pharmaceutical company. When he heard about a cross university multidisciplinary research project about the welfare of puppies sold in Belgium, he grabbed this opportunity. In 2014, he invested himself in a Joint-PhD at the laboratory of ethology and at the veterinary epidemiology unit at Ghent University and the Integrated Veterinary Research Unit of the department of Veterinary Medicine at Namur University. In April 2019, Pierre-Alexandre joined the Corporate Affairs team of Royal Canin Benelux were he is currently holding the position of Scientific Communications advisor.

Pierre-Alexandre Dendoncker is author of scientific articles, contributed to national and international congresses and is actively partaking in popularisation of various animal science topics.

BIBLIOGRAPHY

1. Peer-Reviewed publications in scientific journals:

Biosecurity and management practices in different dog breeding systems have considerable margin for improvements .

Pierre-Alexandre Dendoncker, Christel P.H. Moons, Steven Sarrazin, Claire Diederich, Etienne Thiry, Tiny De Keuster and Jeroen Dewulf

2018, Veterinary Record 183, 381.

On the origin of puppies:
breeding and selling procedures relevant for canine behavioural development.

Pierre-Alexandre Dendoncker, Tiny De Keuster, Claire Diederich, Jeroen Dewulf and Christel P.H. Moons

2019, The Veterinary Record 184, 710.

From breeding facility to the owners' home:
Health and behavioural quality assessment of puppies.

Pierre-Alexandre Dendoncker, Christel P.H. Moons, Tiny De Keuster, Catharina M.M. Lensen, Jeroen Dewulf, Etienne Thiry and Claire Diederich

Journal of Veterinary Behavior: Clinical Applications and Research (Submitted)

Behaviour is in the eye of the beholder: does breeder type influence puppy behaviour?

Pierre-Alexandre Dendoncker, Tiny De Keuster, Katrien Verschueren, Jeroen Dewulf, Claire Diederich and Christel P.H. Moons

Manuscript in preparation

2. Scientific communications:

Adaptation of an existing behaviour assessment tool for use in puppies [Poster]

Pierre-Alexandre Dendoncker, Catharina M.M. Lensen, Christel P.H. Moons, Tiny De Keuster, Jeroen Dewulf and Claire Diederich

First International Canine Behavior & Genetics meeting, 2015, London (UK)

PupWelfare: Ontwikkeling van een hulpmiddel voor Belgische fokkers [Poster]

Pierre-Alexandre Dendoncker, Tiny De Keuster, Etienne Thiry, Jeroen Dewulf, Catharina M.M. Lensen, Claire Diederich and Christel P.H. Moons

KU Leuven, 7de fokkerij dag, 2015, Leuven (BE)

Canine behavioural assessment:
validation of an adapted questionnaire for use in puppies [Oral Presentation]

Pierre-Alexandre Dendoncker, Catharina M.M. Lensen, Tiny De Keuster, Jeroen Dewulf, Christel P.H. Moons and Claire Diederich

SEEVAD, 3ème Symposium international d'Éthologie vétérinaire, 2015, Paris (FR)

Canine behavioural assessment during the first puppy consultation: Development of screening tools and first experiences [Oral Presentation]
Pierre-Alexandre Dendoncker, Christel P.H. Moons, Tiny De Keuster, Catharina M.M. Lensen, William Zonta, Jeroen Dewulf, Etienne Thiry and Claire Diederich
FNRS, First PhD student day, 2018, Liège (BE)

3. Serialised publications:

Biosecurity measures for dog merchants and canine breeding kennels
Pierre-Alexandre Dendoncker, Hilde de Rooster, Eline Abma, Eline Wydooghe and Jeroen Dewulf
Biosecurity in animal production and veterinary medicine : from principles to practice.
ACCO 2018, p.433-452

100 dingen die je moet weten over je hond
Nadine Braeckman, Annelies Dechamps, Pierre-Alexandre Dendoncker, Joshua Dutré, Nick Truyman, Nico Van de Velde en Bart Verbeelen
Beestig, Hotel Hungaria, Lannoo 2018, p.128

100 dingen die je moet weten over je kat
Nadine Braeckman, Pierre-Alexandre Dendoncker, Joshua Dutré, Nick Truyman, Nico Van de Velde, Bart Verbeelen en Tanya Veyt
Beestig, Hotel Hungaria, Lannoo 2018, p.128

The increasing popularity of dogs has provoked a shift in breeding practices, giving rise to a societal debate on intensive dog breeding. The environment shaped by the breeder plays an important role in the behavioural development of puppies, and adverse situations at the breeder can result in behavioural impairment later in life. Additionally, the environment at the breeder can harbour various pathogens that will impair the puppies' health and welfare or represent a zoonotic risk, and puppies and pregnant dams are more at risk to contract a disease.

The overall goal of this research is to contribute to the knowledge base of puppy husbandry practices and selling practices in Belgium, the variation between different breeder types, and the effects thereof on puppy health and behaviour.

To assess all factors that influence the behavioural development and the health of puppies during their time at the breeding facility, Pierre-Alexandre Dendoncker, under the supervision of Professors Christel Moons and Tiny De Keuster (Laboratory of Ethology, Ghent University), Jeroen Dewulf (Veterinary Epidemiology Unit, Ghent University), Claire Diederich (Integrated Veterinary Research Unit, Namur University) and Etienne Thiry (FARAH centre, Liège University) conducted a multidisciplinary research which is the subject of this doctoral dissertation.